EFFECTS OF LEAF LITTER AND ENVIRONMENT ON BRYOPHYTES IN BOREAL FORESTS OF ALASKA

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ABSTRACT

Bryophytes dominate the forest floor, regulate soil microclimate, and participate in the carbon (C) and nitrogen (N) cycles in coniferous boreal forests, while deciduous stands support a lower bryophyte abundance. Recent increases in fire severity throughout the northwestern boreal forest could result in a shift from conifer to deciduous dominated stands. Although broadleaf litter is hypothesized to be detrimental to bryophytes, little is known regarding the mechanisms through which forest canopies affect moss communities and ecosystem processes. In this study, I explored how canopy type and leaf litter affect bryophyte succession, growth, and associated N_2-fixation in interior Alaska. I quantified bryophyte communities in a 163-year chronosequence in deciduous-broadleaf and coniferous stands. Bryophyte communities diverged at about 40 years after fire, with low bryophyte cover in mature deciduous forests and high bryophyte cover in mature coniferous forests, which was associated with increased leaf litter in deciduous stands. I then directly tested leaf litter as the main mechanism explaining this discrepancy in bryophyte abundance between the two forest types using a field-based experiment. I used transplants of a common bryophyte, the feather moss *Hylocomium splendens* (Hewd) Schimp., in deciduous and coniferous stands with manipulations of broadleaf litter to quantify the impacts of leaf litter on moss growth. I found that leaf litter had a much larger detrimental impact on moss growth than the other environmental characteristics of deciduous and coniferous stands, therefore providing experimental support to this previously hypothesized mechanism. Lastly, feather mosses are known to host N_2-fixing cyanobacteria, but little is known regarding how this ecosystem process varies temporally, spatially, or among forest types. I measured N_2-fixation rates in feather mosses over three years using stable isotope labelling ($^{15}N_2$). I found that N_2-fixation rates were extremely variable among years and were linked to precipitation. Feather moss contribution to ecosystem N-budgets remained low in deciduous stands, while they were much higher and variable in coniferous stands. Changes in canopy composition and associated changes in leaf litter inputs induced by climate change will have profound impacts on moss communities, and may have cascading impacts on forest structure and composition.

RÉSUMÉ

Les bryophytes sont des éléments dominants des sous-bois, régulent le microclimat du sol et participent aux cycles du carbone (C) et de l'azote (N) dans les forêts boréales de conifères, alors que les forêts décidues supportent une faible abondance de bryophytes. Une augmentation récente de la sévérité des feux dans le nord-ouest de la forêt boréale pourrait résulter en un changement de forêts dominées par les conifères vers des forêts décidues. Une hypothèse répandue est que la litière de feuilles décidues a un impact négatif sur les bryophytes, mais on connait mal les mécanismes à travers lesquels le couvert forestier et la litière de feuilles affectent les communautés muscinales. Dans cette étude, j'ai exploré la comment le type de couvert forestier et la litière de feuilles influencent la succession, la croissance, et la fixation de N_2 des bryophytes de l'intérieur de l'Alaska. J'ai quantifié les communautés de bryophytes dans une chronoséquence de 163 ans dans des forêts de conifères et de feuillus. Les communautés de bryophytes ont divergé après 40 ans après feu, avec un couvert muscinal faible dans les forêts décidues et élevé dans les forêts de conifères matures, probablement en raison de la litière de feuille dans les forêts décidues. J'ai ensuite testé directement l'impact de la litière de feuilles sur les bryophytes afin d'expliquer cette divergence à l'aide d'une expérience sur le terrain. J'ai utilisé une manipulation de la litière et des transplants d'une espèce commune de bryophyte, la mousse hypnacée *Hylocomium splendens* (Hewd) Schimp. dans des forêts décidues et de conifères afin de quantifier les impacts de la litière sur la croissance des mousses. La litière avait un impact négatif sur les mousses plus important que les autres conditions environnementales associées au type de forêt, fournissant ainsi un support expérimental à un mécanisme précédemment hypothétique. Finalement, les mousses hypnacées sont hôtes de cyanobactéries fixatrices de N_2, mais on en sait peu à propos de la manière dont ce processus varie sous divers couverts forestiers. J'ai mesuré le taux de fixation de N_2 chez les mousses hypnacées durant trois ans à l'aide d'isotopes stables ($^{15}N_2$) comme marqueurs. Les taux de fixation de N_2 étaient extrêmement variables entre les années et étaient liés aux précipitations. La contribution des mousses hypnacées au budget écosystémique de N était faible dans les forêts décidues, alors qu'il était élevé et variable dans les forêts de conifères. Des changements à la composition du couvert forestier et de la litière de feuilles en raison de changements climatiques auront par conséquent de profonds impacts sur les communautés muscinales, et pourraient même avoir des impacts en cascade sur la structure et la composition de la forêt boréale.

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CHAPTER 1: INTRODUCTION

1.1 Bryophytes and plant-soil feedbacks

Plant-soil feedbacks play important roles in community dynamics and ecosystem processes. They occur when plants alter the physical, chemical, and biotic properties of soils in ways that in turn affect plant community composition and structure (Wilson and Agnew 1992, Ehrenfeld et al. 2005, Kulmatiski et al. 2008). These feedbacks can affect ecosystems on multiple scales, ranging from structuring microbial communities to vegetation at the landscape level (Ehrenfeld et al. 2005). Plant-soil feedbacks influence the ecological resilience (Gunderson 2000, Chapin et al. 2010), as well as the successional patterns and processes of terrestrial ecosystems (Kardol et al. 2006, Kulmatiski et al. 2008). Ecological resilience is the capacity of a system to recover following disturbance and maintain its fundamental plant-soil feedbacks, processes, and population structures (Holling 1973, Gunderson 2000). Climate change impacts on temperature, precipitation, and disturbance are likely to alter plant-soil feedbacks, and thereby indirectly alter ecosystem function at multiple scales (Putten et al. 2013, IPCC 2013).

Plant-soil feedbacks are highly influenced by ground covering bryophytes, a group of non-vascular plants that is composed of mosses, liverworts, and hornworts, because they are situated at the interface between above and belowground processes (Lindo et al. 2013). Bryophytes have a worldwide distribution and are commonly found in all biomes, including Arctic and Antarctic tundra (Chapin et al. 1991, Longton 1997), glaciers (Coulson and Midgley 2012), deserts (Belnap 2003), aquatic systems (Carpenter and Lodge 1986), and forests in the boreal (Turetsky et al. 2012), temperate, and tropical zones (Bates and Farmer 1992). The importance of ecosystem processes carried out by bryophytes is often disproportionate to the fraction of living biomass they represent (Sveinbjörnsson and Oechel 1992), and they are often considered as keystone species (Rochefort 2000, Frego 2007, Turetsky et al. 2010). Bryophytes can contribute significantly to aboveground productivity and carbon (C) storage, nutrient cycling, soil temperature regulation, moisture retention, and have facilitative or competitive interactions with vascular plants (Turetsky 2003, Cornelissen et al. 2007). Many species of bryophytes host dinitrogen (N_2) fixing cyanobacteria, which may play a significant role in N-cycling in nutrient poor ecosystems (During and Tooren 1990). In addition, many life-history traits of bryophytes (e.g. capacity to grow on bare substrates, tolerance for drying, small wind-dispersed spores, and clonal reproduction) make them excellent pioneer species (Bates and

Farmer 1992). Therefore, environmental changes that impact bryophyte communities will likely cascade to cause impacts on the plant-soil feedbacks in which bryophytes are involved (Chapin et al. 2000, Beringer et al. 2001, Cornelissen et al. 2007).

Bryophytes play a particularly important role in the circumpolar boreal forest where mosses are ubiquitous (Turetsky et al. 2012). Boreal forests have historically acted as a carbon sink, containing about 30% of terrestrial carbon in their soils (Myneni et al. 2001, McGuire et al. 2009, Deluca and Boisvenue 2012). About 80% of C stored in boreal organic soils has been produced by bryophytes (Apps et al. 1993, Kolari et al. 2006). In coniferous boreal forests, feather mosses, large pleurocarpous bryophytes that are characteristic of circumpolar boreal forests (Bonan and Shugart 1989, Esseen et al. 1997), can account for 20-40% of total net primary production (Goulden and Crill 1997, Bond-Lamberty et al. 2004, Turetsky et al. 2010). This is particularly true for black spruce forests, one of the dominant boreal forest types, where extensive moss carpets composed of feather mosses (e.g., *Hylocomium splendens* (Hewd) Schimp., *Pleurozium schreberi* (Brid.) Mitt., and *Ptilium crista-castrensis* (Hewd.) De Not.) usually cover the ground in black spruce (*Picea mariana* (Mill) B.S.P.) stands (Van Cleve et al. 1983a). In these forests N is often limited and biological N_2-fixation, possibly conducted by moss dwelling cyanobacteria, is one of the most important ecosystem processes in nutrient cycling (Tamm 1991, Cleveland et al. 1999, DeLuca et al. 2002). The presence or absence of bryophytes, and their cover and species composition, could provide key ecosystem processes in different boreal forest types (Johnstone et al. 2010a, Turetsky et al. 2012).

1.2 Bryophyte community development in alternate boreal canopy types

The boreal forest is a mosaic composed not only of coniferous stands where bryophytes are abundant, but also of broadleaf deciduous and mixed-wood stands where bryophytes are less abundant (Hart and Chen 2006). In interior Alaska, two major vegetation types dominate the forest landscape: evergreen conifer stands of black spruce, which account for an estimated 39% of the landscape, and deciduous broadleaf stands dominated by aspen (*Populus tremuloides* Michx.) or birch (*Betula neoalaskana* Sarg.), which account for about 24% of the landscape (Calef et al. 2005). The remainder of the landscape is composed of stands dominated by mixed deciduous and coniferous trees (6%) or white spruce (10%, *Picea glauca* (Moench) Voss), or

non-forested tundra (27%) (Yarie and Billings 2002, Calef et al. 2005). Following large stand-replacing fires, the two main stand types, coniferous black spruce and deciduous aspen or birch, usually self-regenerate, indicating high resilience to fire disturbance (Chapin et al. 2010, Johnstone et al. 2010a, Johnstone et al. 2010b). The structure and composition of the boreal forest in interior Alaska has remained relatively stable over the past 6000 years (Chapin et al. 2010). Bryophyte communities, however, tend to follow directional succession in coniferous forests, with gradual species replacement over time (Foster 1985, Fenton and Bergeron 2006, Turetsky et al. 2010, Fenton and Bergeron 2013). Specifically, colonization, competition and facilitation are known to be important in shaping bryophyte succession within coniferous forests (Foster 1985, Rydin 1997, Fenton and Bergeron 2006, Turetsky et al. 2010, Fenton and Bergeron 2013). Less is known regarding the development of moss communities in deciduous forests where they are less abundant. It is known that canopy composition is a critical driver of species composition in understory communities (De Grandpré et al. 1993, Hart and Chen 2006), but some questions still remain. Does the development of bryophyte communities through post-fire succession depend principally on environmental conditions that can be static or slow changing, or is it primarily controlled by the interactions with the tree canopy, thus creating potential for very rapid changes? In other words, to what degree are plant-plant and plant-soil interactions likely to be essential components of forest resilience and responses to environmental change given the important ecosystem processes carried out by bryophytes?

Moss abundance, species composition and successional trajectories are influenced by environmental conditions, some of which are induced by the development of the tree canopy. Bryophytes are poikilohydric, which means that they can tolerate desiccation by becoming physiologically inactive, and then repair their cells upon rewetting (Glime 2007). Therefore, they are strongly dependent on moisture availability (Glime 2007). Black spruce stands provide favourable conditions to bryophyte establishment and growth as they are mostly found on wet and cold north-facing slopes with permafrost (Van Cleve et al. 1983a, Chapin et al. 2006b). High soil moisture is further promoted by the low rate of evapotranspiration of black spruce trees (Bonan 1991), and the poor drainage caused by shallow active layers and thick organic layers (O'Donnell et al. 2009, Jorgenson et al. 2010; Figure 1.1). Spruce stands also provide shaded areas at the base of mature trees where temperature extremes are ameliorated and where throughfall provides nutrients for bryophytes (Tamm 1953, Foster 1985). In contrast, aspen

stands occupy well-drained south-facing slopes with deep active layers, and birch stands, also found in deep active layer sites, are more common on colder and wetter east- and west-facing slopes (Chapin et al. 2006b). Boreal forests dominated by deciduous broadleaf trees are characterized by warmer and drier soils with high light transmission and shallow organic layers, all of which are hypothesized to be unfavourable to bryophytes (Brown 1963, French 2007, Johnstone et al. 2010a; Figure 1.1). The high evapotranspiration rates of deciduous trees may also deplete soil moisture (Bonan 1991) and potentially restrict the growth of moisture-dependent mosses. Warmer conditions in deciduous stands stimulate relatively high rates of decomposition and nutrient turnover, given sufficient moisture, which favour competitive species capable of fast growth such as deciduous trees that may outcompete mosses (Figure 1.1).

Direct plant-plant interactions with the tree canopy, may also drive patterns of bryophyte abundance. High inputs of broadleaf deciduous leaf litter is the main mechanism put forward to explain the discrepancy in moss abundance between deciduous and coniferous forests (e.g., Van Cleve et al. 1983b, Oechel and Van Cleve 1986, Sveinbjörnsson and Oechel 1992, Hart and Chen 2006, Barbier et al. 2008), even if environmental conditions could otherwise potentially support moss growth (Johnstone et al. 2010a). Indeed, early successional stages dominated by deciduous shrubs that shed abundant leaf litter tend to have a low cover of feather mosses (Oechel and Van Cleve 1986, Turetsky et al. 2010). In mature deciduous stands, mosses are usually found on fallen logs, tree stumps on topographically raised areas from which leaf litter is blown off (Sveinbjörnsson and Oechel 1992), or under gaps in the tree canopy (Jonsson and Esseen 1990). Even though there are numbers of observations supporting this idea, it has rarely been formally tested in an experimental setting (but see Startsev et al. 2008).

There are several ways through which deciduous leaf litter can affect mosses. Broad deciduous leaves tend to remain on top of the moss layer, while smaller coniferous needles tend to fall through the moss layer (Sveinbjörnsson and Oechel 1992). Relatively heavy pulses of leaf litter inputs may form a physical barrier to moss growth, as the slow growth and prostrate form of mosses make them susceptible to burying (Van Cleve et al. 1983b, Beatty and Sholes 1988, Startsev et al. 2008). By forming a layer on top of the mosses, leaf litter can also decrease light availability. Mosses are usually shade tolerant species (Oechel and Sveinbjörnsson 1978, Sulyma and Coxson 2001), but addition of even a single layer of leaves has been shown to decrease moss growth and increase mortality (Startsev et al. 2008). Leachates from leaf litter may contain

inhibitory chemicals, such as phenols, that have allelopathic effects on moss (Légaré et al. 2005, Startsev et al. 2008). Deciduous leaf litter is high in base cations and easily decomposed (Startsev et al. 2008, Melvin et al. 2015), and may therefore increase nutrient availability in ways that reduce the competitive ability of moss against vascular plants (Messier et al. 1998, Côté et al. 2000, Turetsky et al. 2012) or against parasitic moss fungi (During and Tooren 1990, Davey and Currah 2006). Lastly, leaf litter may also have positive impacts on moss communities. Nutrient released from the leaf litter could directly benefit mosses (Oechel and Van Cleve 1986, Sveinbjörnsson and Oechel 1992). In subarctic forests of northern Sweden, rates of N_2-fixation associated with feather mosses increased following moderate additions of birch (*Betula pubescens* Ehrh.) leaf litter (Sorensen and Michelsen 2011). Understanding how interactions between canopy traits (mostly deciduous broadleaf litter production) and stand-associated environmental conditions affect bryophyte abundance and productivity is important in order to predict how changes in forest composition and climate will affect the structure and function of boreal forests.

1.3 Bryophyte-mediated feedbacks in interior Alaska

Feedbacks associated with deciduous and coniferous black spruce stands favour their long-term maintenance and stability on the landscape (Figure 1.1). Bryophyte functional traits promote environmental conditions that are favourable to the stability of black spruce stands in the boreal forest (Johnstone et al. 2010a, Turetsky et al. 2012; Figure 1.1). There, bryophytes, mostly feather mosses, create extensive carpets and build thick organic layers that can be 20-40 cm deep. Organic layers formed from moss have a low bulk density and a high water retention capacity, which favour moist soils (Turetsky 2003, Turetsky et al. 2012) and enhance soil insulation, thus maintaining shallow active layers and cool soil temperatures (O'Donnell et al. 2009, Soudzilovskaia et al. 2013; Figure 1.1). Moist conditions in thick organic moss layers limit the potential for combustion during fires, which increases the likelihood of low severity fire that generate mats of charred surface organic layers after a fire occurs (Viereck et al. 1983, Miyanishi and Johnson 2002, Chapin et al. 2004). Partially-burned layers of soil organic matter are porous materials with a low albedo that dry much faster than mineral soil and are therefore a poor seedbed (Dyrness and Norum 1983, Johnstone and Chapin 2006, Greene et al. 2007). Black

spruce can overcome the recruitment limitations of a poor seedbed by releasing large numbers of relatively large seeds from their semiserotinuous cones after fire, therefore ensuring that some seeds will land in a suitable site for germination and establishment (Zasada et al. 1992, Johnstone and Chapin 2006, Greene et al. 2007). Moss recolonization is usually rapid after low severity fires, i.e. fires that do not burn deep into the soil organic matter, and can occur from unburned patches, fragments, or spores (Benscoter 2006, Shetler et al. 2008). Overall, black spruce-feather moss stands favour high moss net primary production, accumulation of thick organic layers, and recurring dominance of black spruce after fire (Fenton et al. 2005, Hollingsworth et al. 2006, Johnstone et al. 2010a; Figure 1.1).

Deciduous broadleaf stands dominated by aspen or birch are characterized by warm, dry soils, high light transmission, and high nutrient availability (Figure 1.1). Moreover, annual production of abundant leaf litter is expected to be one of the main mechanisms preventing or excluding moss establishment in those stands (Johnstone et al. 2010a), thus maintaining environmental conditions hypothesized to be unfavourable to mosses. When deciduous stands burn, their dry and shallow organic layers are likely to burn down to the mineral soil (Miyanishi and Johnson 2002) on which deciduous species can establish from seed. Moreover, pre-fire deciduous trees can resprout if the belowground biomass, such as roots, remained protected during fire of lower severity (Schimmel and Granström 1996, Johnstone and Chapin 2006, Greene et al. 2007; Figure 1.1). Plant-soil feedbacks associated with leaf litter production are favouring self-replacement in those stands following fire.

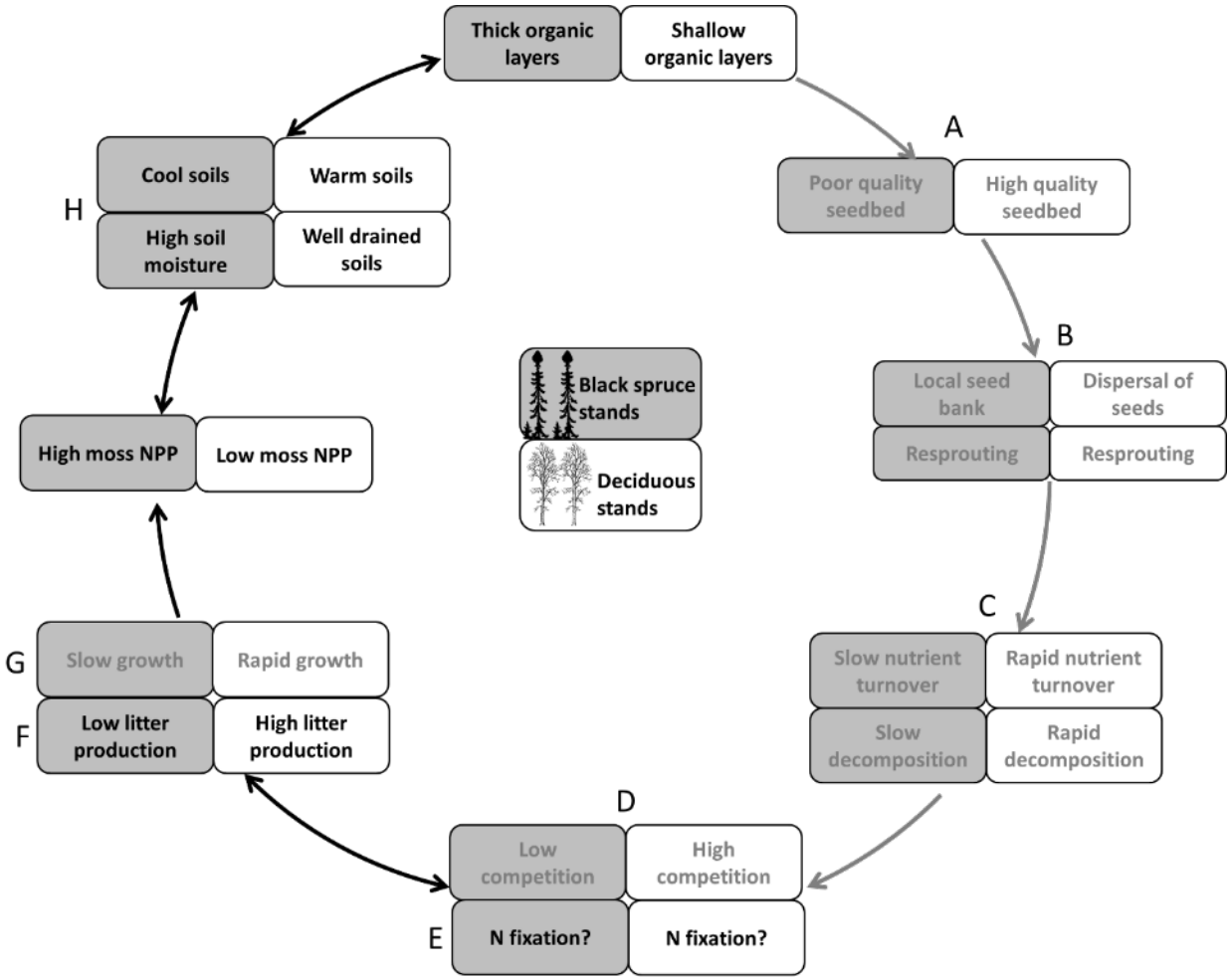

Figure 1.1: A conceptual diagram illustrating parallel feedbacks between community composition and ecosystem processes that are hypothesized to stabilize forest cover within alternate forest types dominated by black spruce (grey boxes) or deciduous broadleaf trees (white boxes). Arrows in the cycles represent processes. Black arrows and fonts indicate processes and elements that I investigated in this project. Grey arrows and fonts indicate processes that are not investigated in this project. Letters refer to previous work that can inform those processes (the list of references is not exhaustive). A. Johnstone and Chapin (2006), Greene et al. (2007). B. Schimmel and Granström (1996), Greene et al. (2007). C. Melvin and Mack (unpublished data). D. Hart and Chen (2006). E. DeLuca (2002), Gundale et al. (2009). F. Ahlgren and Ahlgren (1981), Van Cleve and Viereck (1981), Startsev et al. (2008), G. Fenton et al. (2005), Hollingsworth et al. (2006), Johnstone et al. (2010a). H. Elumeeva et al. (2011), Soudzilovskaia et al. (2013). Figure modified from Johnstone et al. (2010a).

1.4 Climate change and disturbance

Ecosystem responses to recent climate change have been reported from every continent, and substantial warming has been documented in many Arctic and alpine locations (IPCC 2013). Northern latitudes are predicted to experience some of the fastest changes in climate throughout the next century (ACIA 2005, IPCC 2013). Climate change will have both direct and indirect impacts on moss communities. Warmer temperatures and altered precipitation regimes may lead to an increase in the frequency of drying and rewetting cycles or an increase in prolonged droughts, which may hinder moss productivity (Longton 1992, Rousk et al. 2014). Vascular plants will also present direct responses to environmental conditions, which will then indirectly affect mosses through their litter or competitive interactions (Longton 1992, Sveinbjörnsson and Oechel 1992). Impacts of climate change on disturbance regimes will alter vascular plant successional patterns, which will then indirectly affect moss communities.

Recent warming has been associated with increases in fire severity (Kasischke and Turetsky 2006), extent (Flannigan et al. 2005) and frequency (Balshi et al. 2009) in north-western boreal forests. Higher severity fires involve a deeper combustion of the moss and organic soil layers, which increases the amount of exposed mineral soil following fire (Johnstone and Chapin 2006). This increase in exposed mineral soil has led to a shift in canopy dominance from black spruce to deciduous forests dominated by either Alaska paper birch or trembling aspen (Johnstone et al. 2010b). This is because on high quality mineral soils, small-seeded and wind-dispersed deciduous species, such as trembling aspen and Alaska paper birch, are able to establish and have a competitive advantage over black spruce (Johnstone and Chapin 2006, Greene et al. 2007). In Alaskan boreal forest, initial tree recruitment in the first few years after fire determines future mature canopy dominance (Johnstone et al. 2004). Thus, once deciduous stands have established, their plant-soil feedbacks are hypothesized to maintain low feather moss abundance and hence shallow organic layers, and have the potential to promote resilience of these stands, making the likelihood of return to a black spruce state very low (Johnstone et al. 2010a).

An increase in deciduous tree abundance following changes in the fire regime is expected to decrease moss abundance through augmented litter inputs or through suboptimal environmental conditions, for example, changes in moisture or nutrient availability (Chapin et al.

2004, Hart and Chen 2006, Johnstone et al. 2010a). However, it is still unclear what the quantitative impacts of deciduous broadleaf vs. coniferous canopies and leaf litter are on growth, survival, species composition, and biomass accumulation of mosses (Turetsky et al. 2010, Turetsky et al. 2012). N_2-fixation associated with boreal mosses may also be affected by litter inputs or the different environmental conditions prevailing in deciduous stands, although this has not been investigated (DeLuca et al. 2008, Gundale et al. 2009). Understanding how canopy composition, leaf litter and bryophytes interact is a critical piece missing to support the proposed plant-soil feedbacks in boreal forests and to allow for predictions of changes in bryophyte driven ecosystem processes under a changing climate.

1.5 Research objectives and dissertation structure

The overarching goal of this thesis is to evaluate the respective impacts of environmental conditions and canopy type through leaf litter inputs on bryophyte postfire succession, as well as on their ecosystem processes such as N_2-fixation, and biomass production.

Although postfire succession of boreal canopy species has been well studied (e.g., (Heinselman 1981, Foster 1985, Bergeron and Dubuc 1988, Chapin et al. 2006), it remains unclear how successional patterns of forest understory species, and particularly bryophytes, correspond to patterns of forest canopy types over time. I assessed how bryophyte abundance and species composition changed along a successional chronosequence of 163 years in stands representing the three main successional pathways of Alaskan boreal forests, i.e. in trembling aspen, Alaskan paper birch, and black spruce (Chapter 2). I analyzed bryophyte species composition and environmental variables (e.g. organic layer depth, moisture, leaf litter cover) from 83 stands in order to assess how and when bryophytes communities changed over time in the three forest types. This unique dataset allowed me to identify a clear time scale of changes in bryophyte communities, and to pin point potential environmental drivers. I hypothesized that, because of the suggested role of leaf litter, bryophytes communities in deciduous and coniferous forests may follow divergent successional trajectories, as increasing leaf litter inputs in deciduous stands may lead to the exclusion of colonizing species and prevent establishment of later succession bryophytes, such as feather mosses.

I then tested whether differences in moss abundance and productivity among forest types was due to environmental variables, or to direct canopy effects through leaf litter inputs (Chapter 3). I used field-based experiments with moss transplants to test the relative importance of leaf litter vs. stand differences in other environmental factors as mechanisms driving moss abundance and growth among deciduous and coniferous stands. I quantified the impact of deciduous leaf litter and site conditions on feather moss growth, health (greenness), reproductive output (sporophyte production), and stand-level biomass production. Experimental evidence directly addressing the underlying mechanisms in plant-associated feedbacks is rare. My contribution of the understanding of the interaction between mosses and leaf litter in deciduous and coniferous stands is therefore novel. I hypothesized that the shading, crushing, and chemical impacts of leaf litter would have significant detrimental impacts on moss growth, health, reproductive output, and biomass production, and that this effect should be relatively more important than that of environmental conditions. Moss productivity should be higher in spruce than in birch stands given the more suitable environmental conditions and the higher moss abundance.

Lastly, I explored how variations in bryophyte composition and abundance among forest types may have important implications for a key ecosystem process: N_2-fixation (Chapter 4). I estimated the contribution of cyanobacteria-based N_2-fixation occurring in common feather mosses in the Alaskan boreal forest and how this varied among coniferous and deciduous forest types, over the growing season, and across a nutrient availability gradient. Most of the work conducted on N_2-fixation in boreal ecosystems has used an indirect method for estimating fixation rates (acetylene reduction). I used enrichments with the stable isotope of N to accurately and directly measure rates of moss-associated N_2-fixation in three pairs or black spruce and Alaska paper birch stands and paired this with a precise stand level N budget to estimate annual contributions of feather moss associated N_2-fixation to N fluxes and pools. I hypothesized that the conditions in spruce stands (high moss cover, high moisture, low N availability) should be much more suitable to high N_2-fixation rates and to total N_2 fixed than in birch stands (low moss cover, low moisture, high N availability and leaf litter inputs). Moreover, given that sampling took place over three very different years in terms of weather conditions, I expect that cooler and wetter summers should lead to higher rates of N_2-fixation.

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CHAPTER 2: PATTERNS OF BRYOPHYTE SUCCESSION IN A 160-YEAR CHRONOSEQUENCE IN DECIDUOUS AND CONIFEROUS FORESTS OF BOREAL ALASKA

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I led the 2013–2015 field and laboratory work, data analysis, and drafted the manuscript with guidance from Heather D. Alexander, Michelle C. Mack, and Jill F. Johnstone. This paper provides the basis for investigating broad scale impacts of canopy traits and leaf litter on bryophyte abundance and post-fire succession in boreal forests. Michelle C. Mack and Jill F. Johnstone developed the field methods and initiated the sampling; Heather D. Alexander led the 2008–2010 field and laboratory work; Michelle C. Mack and Jill F. Johnstone provided advice on data collection and research questions; and all authors provided input to this manuscript. Mid- and late-succession data on bryophyte species composition and site environmental variables were shared by Heather D. Alexander and Michelle C. Mack.

2.1 Introduction

In high-latitude ecosystems such as boreal forests, bryophytes (mosses, liverworts, and hornworts) are dominant and ubiquitous components of the understory and account for a significant proportion of plant diversity (Turetsky et al. 2012). Boreal ecosystems are also characterized by large stand-replacing fires, which trigger successional changes in bryophyte communities. General patterns of postfire bryophyte regeneration have been investigated in coniferous stands (Foster 1985, Turetsky et al. 2010, Fenton and Bergeron 2013), in which colonization, competition (Rydin 1997) and facilitation (Fenton and Bergeron 2006) are known to be important in shaping bryophyte succession. However, the boreal forest is a mosaic also composed of mixedwood and deciduous stands in which bryophytes are less abundant, and bryophyte succession is poorly documented (Hart and Chen 2006). The roles of bryophytes have often been overlooked in ecosystem studies, even though they can account for a surprisingly large portion of net aboveground primary production and influence regulation of soil microclimate, permafrost stability, nutrient cycling, and ground fuel loads (Turetsky et al. 2012). Knowledge of where, when, and why bryophyte communities change throughout succession is an important gap in our understanding of boreal ecosystem functioning.

Forest canopy composition in boreal forests is one of the major factors influencing understory communities, including bryophytes (De Grandpré et al. 1993, Hart and Chen 2006). Thus, factors that drive changes in canopy composition will likely cause concomitant changes in bryophyte communities. Changes in the fire regime associated with recent climate warming in Alaska and northwestern Canada are predicted to have dramatic impacts on succession, composition, and structure of boreal forest ecosystems (Johnstone et al. 2010a, 2010b). For example, increases in fire severity that more fully combust bryophyte and organic soil layers have caused recruitment of canopy species to shift from dominant conifer species such as black spruce (*Picea mariana* Mill B.S.P.) to alternate deciduous species such as Alaska paper birch (*Betula neoalaskana* Sarg.) and trembling aspen (*Populus tremuloides* Michx.) (Johnstone et al. 2010b). Shifts from conifer to deciduous dominance may affect bryophyte communities because the high inputs of leaf litter and warm, dry soils characteristic of deciduous forests are hypothesized to reduce bryophyte abundance (Van Cleve and Viereck 1981, Hart and Chen 2006). Changing bryophyte communities as a consequence of shifting forest dominance could

affect key processes such as nutrient cycling, soil temperature, and vascular plant recruitment (Hart and Chen 2006).

In this context, understanding bryophyte succession under alternate pathways of canopy succession in boreal forests will provide insight into the possible drivers of the different patterns of composition and ecosystem function found in mature deciduous and coniferous forests (Van Cleve and Viereck 1981, Johnstone et al. 2010a). Currently, it remains unclear whether contrasting bryophyte communities in mature stands develop from diverging or completely distinct successional trajectories in deciduous and coniferous stand types. Distinct successional trajectories could be caused by initial differences that persist through time such as legacies from fire severity, colonization, or site-specific characteristics (Foster 1985, Hart and Chen 2006, Turetsky et al. 2012). Diverging successional trajectories of bryophytes in which community composition is similar early in succession, but forms distinct bryophyte associations as stands age may be caused by canopy-driven changes in processes such as light transmission, evapotranspiration, throughfall, or litter production (Van Cleve and Viereck 1981).

The purpose of this study was to examine patterns of postfire bryophyte succession in boreal forests dominated by coniferous or deciduous trees. We explicitly tested competing hypotheses of distinct or diverging trajectories of succession by testing for differences in bryophyte abundance and species assemblages among forest canopy types in different successional stages. We gathered data on bryophyte communities from stands in interior Alaska that shared similar environmental conditions and prefire history, but ranged in postfire age from 8 to 163 years. We predicted that bryophyte cover and species assemblages would vary with stand age and forest canopy type, but if bryophyte succession followed "distinct" patterns among canopy types, these differences would appear early in succession, whereas "divergent" succession processes would lead to differences in bryophyte cover and composition appearing among canopy types only later in succession. By investigating how patterns of bryophyte abundance change at different time points during succession and looking for overlap or differences both in cover and species composition, we can differentiate between the two types of succession. We used information on stand ages to identify time periods when divergence occurs, if present, and investigated associations of environmental covariates with changes in bryophyte communities to develop hypotheses of important factors shaping patterns of bryophyte succession in deciduous and coniferous forest stands. Results of this study provide insights into

the processes that drive bryophyte community assembly in boreal forests, which is critical in understanding the role of bryophytes in boreal forests in the context of global change.

2.2 Methods

2.2.1 Study area

Bordered to the north by the Brooks Range (~67°N) and to the south by the Alaska Range (~63°N), interior Alaska is characterized by isolated mountain ranges, gently sloping uplands, flat lowlands, and large floodplains around the Yukon, Tanana, and Kuskokwim rivers. Interior Alaska has an extreme temperature range, with temperatures ranging between -70°C and 35°C, a mean annual air temperature of -2.5°C, and a growing season that lasts about 135 days. Approximately 35% of the annual 286 mm of precipitation falls as snow (Hinzman et al. 2005). Interior Alaska is within the discontinuous permafrost zone, and about 75-80% of the ground is underlaid by permafrost (Osterkamp and Romanovsky 1999).

Forests in the uplands of interior Alaska are typically dominated by one of three tree species: black spruce, trembling aspen, and Alaska paper birch. Black spruce stands are the most common forest type in interior Alaska, accounting for an estimated 39% of the landscape (Calef et al. 2005), and are mostly found on wet and cold north-facing slopes with permafrost (Van Cleve et al. 1983a, Yarie and Billings 2002). In black spruce stands, the forest floor is usually covered by extensive bryophyte carpets composed of pleurocarpous (feather) mosses such as *Hylocomium splendens* (Hewd) Schimp., *Pleurozium schreberi* (Brid.) Mitt., and *Ptilium crista-castrensis* (Hewd.) De Not, or by *Sphagnum* spp. (Van Cleve et al. 1983a). Deciduous-dominated stands are estimated to occupy about 24% of interior Alaska, while the remainder of the landscape is principally tundra (27%) or forested by white spruce (*Picea glauca* (Moench) Voss; 10%) (Calef et al. 2005). Aspen stands occupy well-drained south-facing slopes with deep active layers, while birch stands, also found in deep active layer sites, are more common on colder and wetter east- and west-facing slopes (Chapin et al. 2006b). Only about 6% of interior Alaska's boreal forest is composed of mixed stands with both coniferous and deciduous species sharing canopy dominance (Yarie and Billings 2002). While these different forest types vary in their preferred habitat, there is a considerable amount of overlap in their ecological tolerances

(Chapin et al. 2006b), allowing for changes in canopy composition triggered by disturbance such as fire or dispersal events (Johnstone et al. 2010a, 2010b).

Differences in fire severity in black spruce stands can lead to the establishment of black spruce, trembling aspen, or Alaska paper birch postfire stands (Johnstone et al. 2010a, 2010b), thus creating three distinct successional pathways based on canopy dominance. In the boreal forests of western North American, most seedlings establish within a few years after fire and they are strong predictors of the mature canopy composition of the forest (Johnstone et al. 2004) thus canopy dominance appears to be principally determined by initial patterns of direct regeneration (e.g. Ilison and Chen 2009). Moreover, with a fire interval of about 100 years, succession from a deciduous stand to a coniferous stand via relay dominance is rarely completed (Yarie 1981). We checked our assumption of direct regeneration vs. relay dominance by surveying woody debris in all stands and found no evidence of deciduous trees being replaced by later establishment of conifers. We therefore assume that stand age and forest composition do not co-vary within the range of ages that we surveyed.

We used a chronosequence or space-for-time substitution approach to examine postfire bryophyte succession. This type of study assumes that climate, regional pool of organisms, topography, and parent material are held constant across sites and only time after disturbance varies (Walker et al. 2010). All of the stands sampled here had originated from a fire in stands that formerly contained at least some black spruce, confirmed by evidence of dead and charred black spruce trees (Alexander et al. 2012). At the time of sampling, these stands were dominated by black spruce, Alaska paper birch, or trembling aspen. In order to limit other confounding factors, we focused our sampling on north-, east-, and west-facing (or relatively flat) mesic black spruce, Alaska paper birch, and aspen stands and measured a number of environmental covariates, including a soil description. Southeast-facing slopes and poorly drained black spruce stands were avoided as they are usually not dominated by black spruce or have a longer fire cycle, respectively.

2.2.2 Sampling design

We surveyed a total of 83 stands ranging in age from 8 to 163 years in upland boreal forests in interior Alaska between 2008 and 2015. The stands were stratified by time since fire and forest type; stands burned within the fire history record (> 1945) were located within 32

different areas burned by a single fire event and mature forests were sampled in surrounding areas that had not burned since 1945 (Table 2.1, Table A1.1). Burned areas were mapped by the Alaska Fire Service, and their locations were acquired from the Alaska Geospatial Data Clearinghouse (U.S. Department of the Interior Bureau of Land Management 2012). Where possible, we selected at least one pure black spruce stand and one pure deciduous stand in each burned area (Table A1.1). To minimize travel time between sites, all sites were located > 100 and < 600 m from a road. We conducted extensive sampling within a successional stage for each forest type to enable robust inferences from our chronosequence design (Walker et al. 2010). Our study included 29 stands from early successional forests (8-19 years since fire), 45 stands in mid-successional forests (20-59 years since fire, as per Alexander et al. (2012)), and nine stands in late-successional forests (60-163 years since fire). Early successional forests were sampled in 2013-2014 using the same design as that used in the sampling of mid- to late-successional forests in 2008-2009 (Alexander et al. 2012).

Table 2.1: Number of sites sampled and average site characteristics (with standard errors) according to estimated time since fire (years) and forest canopy type.

	Early succession (8-19 years)			**Mid-succession (20-59 years)**			**Late succession (60-163 years)**		
	Black spruce	Alaska paper birch	Trembling aspen	Black spruce	Alaska paper birch	Trembling aspen	Black spruce	Alaska paper birch	Trembling aspen
Number of sites	7	9	13	21	7	14	5	4	3
Bryophyte cover (%)[a]	69.5 be (12.2)	71.3 de (16.1)	66.3 be (15.1)	52.7 bcd (19.6)	33.9 ac (26.3)	23.5 a (15.5)	87.7 e (15.3)	8.6 a (10.4)	31.7 ab (13.6)
Leaf litter cover (%)	68.3 (4.2)	62.6 (11.3)	74.8 (8.5)	50.9 (17.7)	73.6 (20.3)	75.1 (19.8)	33.8 (13.2)	94.8 (2.5)	88.0 (3.7)
Organic layer (cm)	9.7 (3.5)	9.9 (2.0)	7.6 (3.8)	10.1 (5.1)	8.0 (4.1)	4.9 (2.2)	24.7 (4.3)	8.9 (4.4)	3.8 (1.2)
Spruce basal area ($cm^2\ m^{-2}$)	1.5 (2.0)	0.4 (0.3)	1.1 (1.2)	6.5 (4.5)	2.6 (3.7)	1.4 (1.1)	15.0 (8.2)	2.3 (3.2)	0.2 (0.1)
Birch basal area ($cm^2\ m^{-2}$)	0.2 (0.2)	3.7 (4.1)	0.3 (0.6)	1.0 (1.8)	14.0 (12.6)	0.3 (0.7)	0.4 (0.5)	26.9 (11.5)	2.4 (4.1)
Aspen basal area ($cm^2\ m^{-2}$)	0.1 (0.1)	0.4 (0.8)	1.9 (2.4)	0.7 (1.5)	0.1 (0.1)	8.8 (5.2)	0.0 (0.0)	1.3 (2.7)	31.0 (3.1)
Shrubs basal area ($cm^2\ m^{-2}$)[b]	2.9 (2.4)	1.9 (1.7)	3.7 (3.4)	3.0 (3.4)	1.8 (2.2)	1.8 (2.6)	0.5 (0.5)	3.4 (3.0)	0.9 (1.0)

[a] Averages that do not share a letter are significantly different based on a Tukey HSD post-hoc test ($p < 0.05$) following a two-way ANOVA.
[b] Shrubs includes large deciduous shrubs, i.e. *Alnus* spp., *Salix* spp., and *Betula* spp.

In each stand, we positioned a 100 m long transect perpendicular to the slope or used a random compass bearing if the ground was flat. The methods used to measure stand structure are presented in more detail in Alexander et al. (2012). Basal diameter (BD) and diameter at breast height (DBH) of all living and dead trees and large shrubs were recorded within 1 m on each side of the transect over a 10 m section for every 20 m subsection. In some instances, this area was reduced to allow measurements of very dense early successional stands. In stands > 20 years old, we collected a core or a basal disk from 10 trees of the dominant canopy species to confirm the stand age obtained from fire history maps. Allometric equations from Alexander et al. (2012) and Berner et al. (2015) were used to estimate aboveground biomass of individual trees and tall shrubs (*Salix* spp., *Betula* spp., and *Alnus* spp.). All stands were attributed to a forest type (black spruce, Alaska paper birch, or aspen) based on the tree species with the largest contribution to total woody biomass (Table 2.1, Table A1.1). A deciduous importance value (DecIV) index was calculated based on the contribution of deciduous trees to total stand aboveground biomass (for calculations, see Alexander et al. (2012)). Organic layer thickness (fibric and humic layers), mineral soil texture (only in stands sampled in 2013-2014), and pH were measured every 10 m along the transect. Soil moisture was measured using a ECH2O EC-TM soil moisture probe read by an Em50 data logger (Decagon Devices Inc., Pullman, Washington, U.S.A.) next to the soil sampling core in all the stands sampled by Alexander et al. (2012). Soil moisture was not measured in the early successional stands in 2013 and 2014, but a general drainage class was recorded for each stand (Johnstone et al. 2008). A drainage class was estimated for older stands using topography and soil moisture data. Finally, we recorded slope, aspect, and GPS coordinates of each transect, and calculated a heat load index (McCune and Keon 2002).

We used a 50 × 50 cm grid with 25 equally spaced sampling points to survey the understory plant species composition by point-intercept sampling. All vascular and nonvascular species, as well as bare ground and dead organic material (wood and leaf litter), that touched a pin inserted at each sampling point were recorded. The grid was placed perpendicular to, and 1 m from, the transect every 20 m (five replicates). Cover (%) of bare ground, coarse woody debris, leaf litter, total vascular plants, and lichens were calculated and used as environmental covariates. We did not sample bryophytes that grew on live or dead wood and focused instead on the dominant forest floor taxa. The point-intercept sampling method presents accurate estimation of the abundance of common species but may not allow for the recording of uncommon or rare

species (Mamet et al. 2016, Vanderpoorten et al. 2010). Given that our main interest was linked to understanding how bryophytes affect ecosystem processes, the most important information from community composition would come from common species, and we felt confident that missing rare bryophyte species was not a problem in that regard. Samples of all encountered unknown species were collected for identification in the lab. Bryophyte nomenclature followed Anderson et al. (1990). We identified a total of 106 different taxa in our samples: 67 vascular plant species, 10 taxa of lichens, and 29 taxa of bryophytes (Table 2.2). Within the bryophytes, we recorded taxa from 11 families of true mosses, three families of liverworts and one *Sphagnum* family. We could identify 19 true mosses to the species level and six species of true mosses could only be identified to the genus level. Some unknown bryophytes could not be identified because of the small size or poor quality of the samples. Because of the limited taxonomic resolution, we use the term "bryophyte taxa" here rather than species. Bryophyte taxa were further classified in functional groups of colonizers, pleurocarpous mosses (feather mosses), acrocarpous mosses, liverworts, and *Sphagnum* spp. (Turetsky et al. 2010; Table 2.2). Bryophyte cover was summarized in three forms: total bryophyte cover, cover by functional types, and cover of individual taxa. We resampled understory taxa composition in five stands of varied canopy dominance surveyed by Alexander et al. (2012) in 2015 to make sure that there was no significant observer bias in taxa identification and detection.

Table 2.2: Bryophyte taxa list, functional type, and average percent cover in the forest types and successional stages.

Order	Family	Taxa name	Functional type	Early (0-20 years)			Mid- (20-62 years)			Late (63 years +)		
				PM[a]	BN[a]	PT[a]	PM	BN	PT	PM	BN	PT
Bryales	Aulacomniaceae	*Aulacomnium palustre* (Hedw.) Schwägr.	Acrocarpous mosses	10.3	3.1	1.7	10.8	0.9	0.5	10.6	0.0	0.0
		Aulacomnium turgidum (Wahlenb.) Schwägr.		1.7	0.1	0.4	0.0	0.0	0.0	0.0	0.0	0.0
	Bryaceae	*Bryum* spp.	Colonizer	3.2	2.7	0.1	0.0	0.0	0.1	0.0	0.0	0.0
		Leptobryum pyriforme (Hedw.) Wilson		2.6	2.4	0.1	0.0	0.0	0.0	0.3	0.0	0.0
		Pohlia sp.		0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0
		Ceratodon purpureus (Hedw.) Brid.		34.7	32.5	41.2	0.7	0.0	2.3	0.0	0.0	0.0
Dicranales	Dicranaceae	*Dicranum* spp.[b]	Acrocarpous mosses	0.5	0.1	0.0	0.3	1.4	0.3	0.2	3.5	0.3
		Dicranum sp.		0.0	0.0	0.0	1.4	0.3	0.3	3.5	0.2	0.3
		Dicranum undulatum Schrad. ex Brid.		7.0	9.0	13.0	21.0	7.0	14.0	5.0	4.0	3.0
Hypnales	Amblystegiaceae	*Sanionia uncinata* (Hedw.) Loeske	Pleurocarpous mosses	0.2	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
	Brachytheciaceae	*Tomentypnum nitens* (Hedw.) Loeske		0.0	0.0	0.0	1.1	0.0	0.0	1.0	0.0	0.0
	Hylocomiaceae	*Hylocomium splendens* (Hedw.) Schimp.		0.0	0.0	0.1	5.7	6.3	1.6	42.7	4.6	24.3
		Pleurozium schreberi (Brid.) Mitt.		2.2	1.6	0.2	14.2	18.6	1.8	24.5	0.2	1.1
	Hypnaceae	*Hypnum spp.*[b]		0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
		Hypnum cupressiforme Hedw.		0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Hypnum plicatulum (Lindb.) A. Jaeger		0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
		Hypnum revolutum (Mitt.) Lindb.		0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
	Rhytidiaceae	*Rhytidium rugosum* (Hedw.) Kindb.		0.0	0.0	0.1	0.2	0.0	2.1	0.0	0.0	0.0
		Hypnales[b]		0.3	0.1	0.1	0.04	0.3	0.0	0.0	0.0	0.0
	Amblystegiaceae	*Amblystegium* sp.		0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Drepanocladus sp.		0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Order	Family	Taxa name	Functional type	Early (0-20 years)			Mid- (20-62 years)			Late (63 years +)		
				PM	BN	PT	PM	BN	PT	PM	BN	PT
Hypnales	Brachytheciaceae	*Brachythecium* sp.	Pleurocarpous mosses	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
	Thuidiaceae	*Thuidium abietinum* (Hedw.) Schimp.		0.0	0.0	0.0	0.04	0.0	0.0	0.0	0.0	0.0
	Hypnaceae	*Ptilium crista-castrensis* (Hedw.) De Not.		0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0
Polytrichales	Polytrichaceae	*Polytrichum commune* Hedw.	Colonizer	14.3	19.5	6.1	16.1	12.1	14.3	7.0	0.0	0.0
		Polytrichum juniperinum Hedw.		13.7	10.9	29.8	0.00	0.0	0.0	0.0	0.0	0.0
		Polytrichum piliferum Hedw.		0.0	2.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Polytrichum strictum Menzies ex Brid.		0.2	1.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Jungermanniales		Jungermaniales[b]	Liverwort	1.3	0.5	0.2	0.0	0.0	0.0	2.4	0.0	0.0
	Myliaceae	*Mylia* sp.		0.0	0.0	0.0	0.0	0.0	0.0	1.4	0.0	0.0
	Jungermanniaceae	*Lophozia ventricosa* (Dicks.) Dumort. cf.		1.3	0.5	0.2	0.0	0.0	0.0	1.0	0.0	0.0
		Unknown liverworts		0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Marchantiales	Marchantiaceae	*Marchantia polymorpha* L.		3.9	9.5	0.0	0.0	0.0	0.1	0.0	0.0	0.0
Sphagnales	Sphagnaceae	*Sphagnum* spp.	*Sphagnum*	1.4	0.6	1.4	6.6	1.5	0.0	29.4	0.0	1.4
		Unknown moss 1	Unknown mosses	0.0	0.0	0.0	0.1	0.2	0.1	0.0	0.0	0.0
		Unknown moss 2		0.0	0.0	0.0	0.0	0.0	1.2	1.2	1.3	3.2
		Unknown moss 3		0.0	0.0	0.0	3.1	4.0	0.8	3.0	0.6	4.8
		Unknown moss 4		0.0	0.0	0.0	0.8	3.1	0.0	0.0	0.0	0.0

[a] Stands types: PM, *Picea mariana*; BN, *Betula neoalaskana*; PT; *Populus tremuloides*.

[b] Indicates species group including the following rare bryophyte taxa (offset in the table). These groups were used in the ordination analyses rather than rare species and were not included when assessing the total number of identified bryophyte taxa.

2.2.3 Statistical analyses

2.2.3.1 Analyses of bryophyte abundance

All of our analyses were conducted in R 3.1.2 (R Core Team 2016) using the package *vegan* (Oksanen et al. 2016). We were interested in testing whether patterns of bryophyte succession in different forest successional trajectories were similar when considering *(i)* total bryophyte cover, *(ii)* functional group cover, and *(iii)* taxa composition. Using different levels of precision allowed us to make stronger inferences about the successional patterns observed (Walker et al. 2010). We did not include burn area or spatial region as a random effect in our analyses because spatial arrangement was partially confounded with age (sampling within discrete burned areas) and we expected age effects to supersede spatial effects on bryophyte communities. Patterns of total bryophyte, functional group, and individual taxa cover were compared among forest types (black spruce, birch, and aspen) and successional stages (early, mid-, and late succession).

Total bryophyte cover, as well as the three main functional types (colonizers, pleurocarpous mosses, and acrocarpous mosses) were analyzed using a two-way ANOVA (stand type and successional stage) with a type III sum of squares for unbalanced data and followed by a Tukey honest significant difference (HSD) post-hoc test. We assessed differences in bryophyte cover among the three canopy types within each successional stage based on whether 95% confidence intervals were overlapping. Data for functional groups and taxa cover did not meet ANOVA assumptions because of the large number of zero values, so ANOVAs using permutation tests were used (4999 permutations: Anderson and Legendre 1999).

We used a PerMANOVA (multivariate analysis of variance by permutations using a distance-based RDA) adapted for an unbalanced design with 4999 permutations to compare taxa assemblages among forest types and successional stages to patterns observed under random sorting (Anderson and Legendre 1999). The Bray Curtis distance was used on taxa composition data as it is an ecologically appropriate distance measure (Anderson and Legendre 1999). Multivariate dispersion differed between the different successional stages but not among forest types. This is a well-recognized issue with multivariate analyses, which can lead to confusion between within-group variation (dispersion) and mean values of the groups. PerMANOVA is thought to be less sensitive to this issue than some of its alternatives (analysis of similiarities

[ANOSIM] or multi-response permutation procedure [MRPP]) (Anderson 2001). When a significant interaction was found, we conducted a series of one-way PerMANOVAs within each age category, followed by a Tukey HSD post-hoc test.

To support the PerMANOVAs conducted on taxa composition data, we conducted a visual analysis of taxa composition using a nonmetric multidimensional scaling (NMDS) ordination that was conducted on taxa data from the 83 stands using the Bray Curtis distance (McCune et al. 2002). The best solution was selected from 20 independent runs with 200 iterations per run. The number of ordination axes was determined by comparing stress and dimensionality against randomized outcomes (McCune et al. 2002). This analysis was used to detect the main patterns in taxa composition among different forest types and different successional stages.

2.2.3.2 Analyses of environmental covariates

We used two different approaches to investigate how patterns of bryophyte cover were related to measured environmental covariates: univariate and multivariate regression trees, and vector overlays on the NMDS ordination. Analyses were conducted in R (R Core Team 2016) using the packages *vegan* (Oksanen et al. 2016) and *mvpart* (Therneau and Atkinson 2013). Ecologically relevant environmental variables (percent cover of bare ground, coarse woody debris, leaf litter, vascular plant, and lichen; DecIV index; basal area of black spruce, birch, aspen and large shrubs; elevation; heat load index; and moisture class) were selected to minimize collinearity.

Univariate and multivariate regression (MRTs) trees were the main tools used to investigate the relationship between bryophyte cover or composition and environmental covariates. Regression trees are nonparametric methods that are robust to issues associated with ecological data such as non-normality and heteroscedasticity and make no assumptions with regards to the form of the relationship between response variables and explanatory covariates (De'ath and Fabricius 2000). MRTs partition the dataset recursively into subsets in order to minimize within-group heterogeneity, providing a clustering-like result and a dichotomous classification key (McCune et al. 2002). Percent cover of functional groups (six groups) and taxa cover (25 taxa, includingd unknown species) were transformed using the Bray Curtis distance (83 stands) prior to analysis. No transformations were applied to environmental covariates. We

excluded stand age and categories of forest type from these analyses to focus on how changes over time in environmental covariates and continuous variables of forest composition were related to bryophytes. This approach allowed us to include information about the co-occurring tree species in the stands and interpret the potential effects of gradients in canopy composition rather than simple canopy dominance. MRT results are described both by their fit, or percent variation explained, which is calculated as the inverse of the relative error (RE), and their predictive accuracy estimated by the cross-validated error (CVRE). This number estimates the probability of misclassifying a new sample and varies from 0 (good predictor) to 1 (poor predictor) (De'ath and Fabricius 2000). For further comparison of variations in leaf litter cover according to forest types and successional stage, we conducted a two-way ANOVA followed by a Tukey HSD post-hoc test.

We used vector overlays on the NMDS ordination to determine how all covariates were related to bryophyte taxa composition. Pearson correlation coefficients between environmental variables and NMDS scores defined the strength of correlations with ordination axes. Significance of the correlation was determined using 999 permutations.

2.3 Results

2.3.1 Part I: temporal changes in bryophyte communities

2.3.1.1 Total bryophyte cover

Bryophyte cover varied according to forest type and the three postfire successional stages (interaction $F_{4,74} = 8.524$, $p < 0.0001$; Figure 2.1; Table 2.1). Bryophyte cover was high in all early successional forest types ($\bar{x}$ = 69%; Figure 2.1; Table 2.1). While total bryophyte cover was reduced to an average of 40% during the mid-successional stage, it was higher in stands dominated by spruce ($\bar{x}$ = 53%) compared with birch or aspen ($\bar{x}$ = 34% and $\bar{x}$ = 23%, respectively; Table 2.1). Bryophyte cover increased in late succession to an average of 88% in spruce stands, much higher than the cover observed in aspen ($\bar{x}$ = 32%) or birch ($\bar{x}$ = 9%) during late succession (Figure 2.1; Table 2.1). However, it is important to note that the time series for birch (87 years) was not as long as for the other tree species. Differences in total bryophyte cover among forest types indicate a divergence in bryophyte cover between coniferous (spruce) and deciduous (birch and aspen) forest types between 20 and 40 years after fire (Figure 2.1).

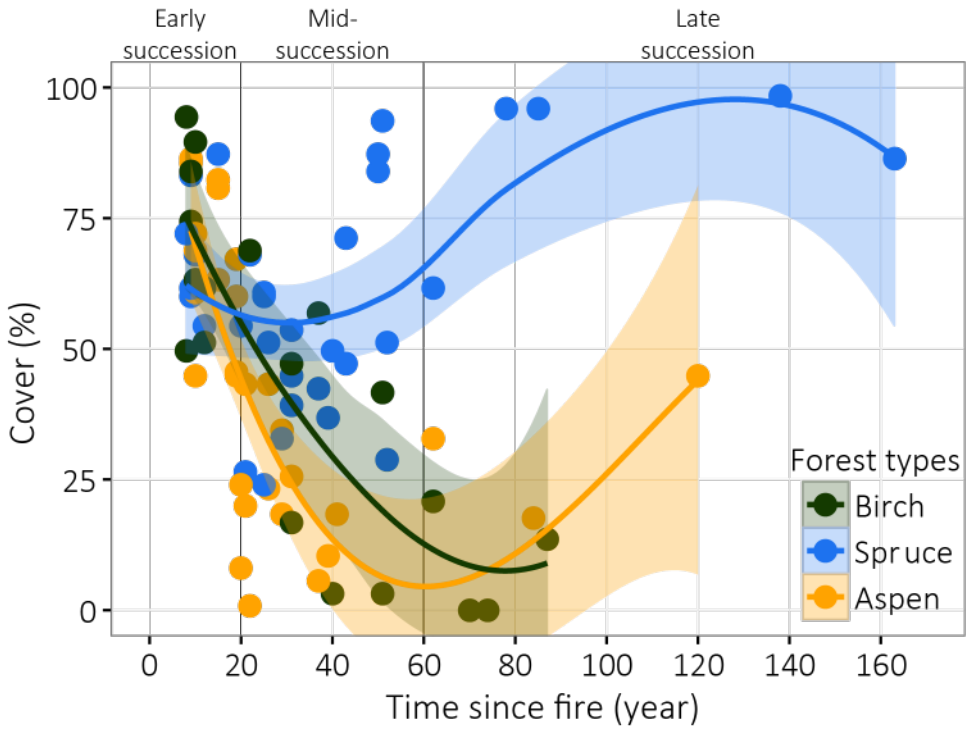

Figure 2.1: Variations in total bryophyte cover (%) versus time after fire in forest stands dominated by black spruce, Alaska paper birch, or trembling aspen. Points represent percent cover for each of the 83 sampled stands, fitted with LOESS (locally weighted scatterplot smoothing) curves with a smoothing parameter of 1 for each series of points. Shadings represent the 95% confidence interval.

2.3.1.2 Functional groups

Colonizer taxa declined quickly from 8 to 40 years, demonstrating a strong effect of successional stage ($F_{2,74} = 91.435$, $p = 0.0002$; Table A1.3a), and followed very similar trends in all forest types (Figure 2.2; Table 2.3). Bryophyte communities in all forest types were dominated by *Ceratodon purpureus* and *Marchantia polymorpha* from 10-20 years after fire and were then dominated by larger acrocarpous taxa such as *Polytrichum commune* and *Polytrichum juniperinum* from 20-40 years after fire (Table 2.2). Pleurocarpous mosses remained low in deciduous stands throughout succession but increased in spruce stands to an average of 63% cover in late succession ($F_{4,74} = 10.111$, $p < 0.0001$; Table 2.3; Figure 2.2; Table A1.3b). Pleurocarpous mosses represented about two thirds of the total bryophyte cover in mature spruce stands. The feather mosses *Pleurozium schreberi* and *Hylocomium splendens* shared the dominance of the pleurocarpous moss cover in all stands (Table 2.2). *Pleurozium schreberi* seemed to be slightly more abundant than *H. splendens* until about 100 years since fire in spruce stands (Table 2.2). The cover of acrocarpous mosses (e.g. *Aulacomnium* spp., *Dicranum* spp.) was higher in spruce stands (about 18%) than in deciduous stands (< 7%) ($F_{2,74}=10.460$, $p = 0.0014$; Table A1.3c), and remained relatively low in all forest types regardless of stand age (Table 2.3; Figure 2.2b). *Sphagnum* spp. cover increased steadily in older spruce stands and was absent from deciduous stands, while liverworts were rare in all forest types at every stage of succession (Table 2.3).

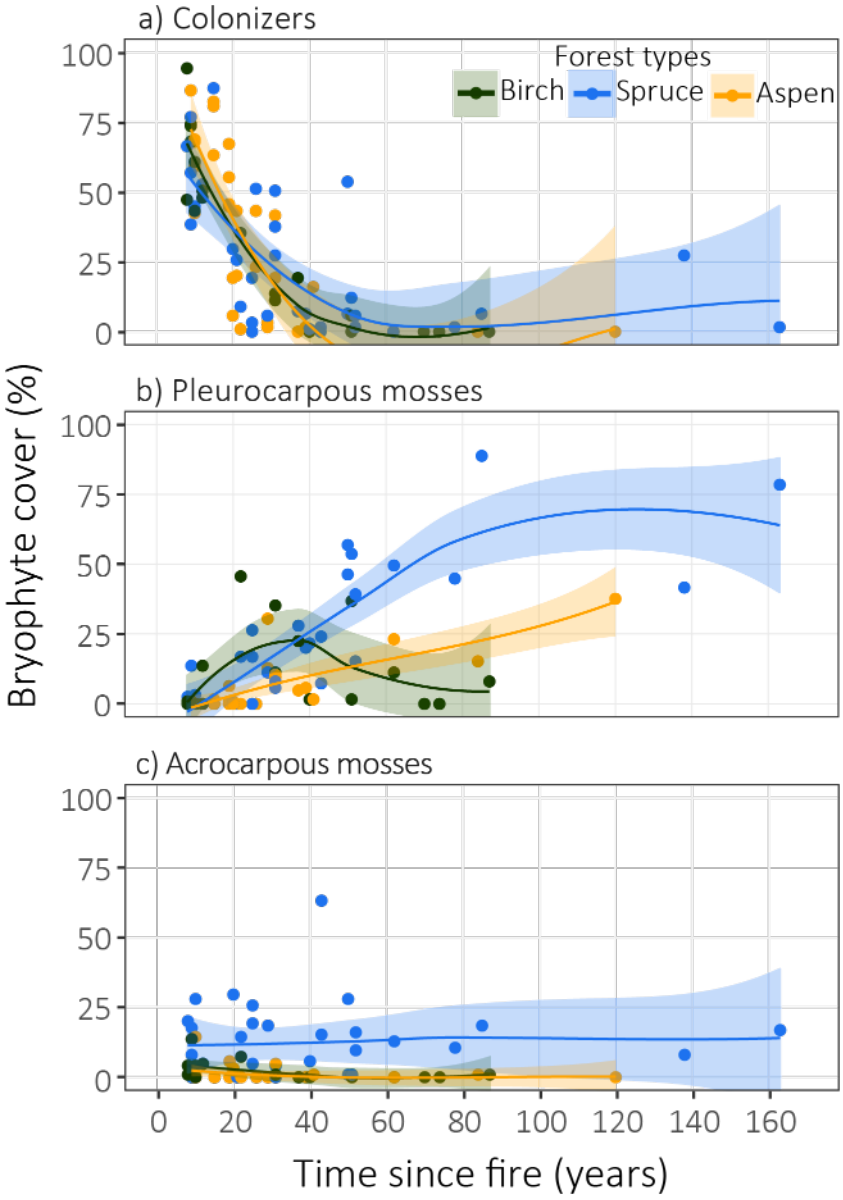

Figure 2.2: Variations in bryophyte cover grouped into functional types of (a) colonizers, (b) pleurocarpous (feather) mosses and (c) other true mosses, plotted against time since fire (years) in spruce, birch, and aspen stands (n = 83). LOESS curves were fit to each series of points with a smoothing parameter of 1. Shadings represent the 95% confidence interval.

Table 2.3: Abundance of bryophytes grouped by functional types and compared according to forest type and successional stage.

Functional group	Successional stage	Forest type	n^a	Average cover (%)	Standard error	Tukey results[b]
Colonizer	Early	Spruce	7	60.5	6.6	
		Birch	9	62.2	5.6	a
		Aspen	13	64.7	4.1	
	Mid	Spruce	21	16.8	4.0	
		Birch	7	12.1	4.7	b
		Aspen	14	17.0	4.3	
	Late	Spruce	5	7.4	5.1	
		Birch	4	0.0	0.0	b
		Aspen	3	0.0	0.0	
Pleurocarpous moss	Early	Spruce	7	2.9	1.9	a
		Birch	9	1.7	1.5	a
		Aspen	13	0.7	0.5	a
	Mid	Spruce	21	19.4	3.8	a
		Birch	7	22.1	6.7	a
		Aspen	14	5.4	2.3	a
	Late	Spruce	5	60.6	9.6	b
		Birch	4	4.8	2.8	a
		Aspen	3	25.3	6.6	a
Acrocarpous mosses	Early	Spruce	7	11.2	4.1	a
		Birch	9	3.7	1.4	b
		Aspen	13	1.8	1.1	b
	Mid	Spruce	21	12.2	3.4	a
		Birch	7	1.3	1.0	b
		Aspen	14	0.8	0.4	b
	Late	Spruce	5	13.3	1.9	a
		Birch	4	0.2	0.2	b
		Aspen	3	0.3	0.3	b
Sphagnum	Early	Spruce	7	1.4	0.9	NA
		Birch	9	0.6	0.6	NA
		Aspen	13	1.4	1.2	NA
	Mid	Spruce	21	6.6	3.4	NA
		Birch	7	1.5	1.4	NA
		Aspen	14	0.0	0.0	NA
	Late	Spruce	5	29.4	16.3	NA
		Birch	4	0.0	0.0	NA
		Aspen	3	0.0	0.0	NA
Liverworts	Early	Spruce	7	5.6	2.8	NA
		Birch	9	10.0	7.1	NA
		Aspen	13	0.2	0.2	NA
	Mid	Spruce	21	0.0	0.0	NA
		Birch	7	0.0	0.0	NA
		Aspen	14	0.1	0.1	NA
	Late	Spruce	5	2.4	1.5	NA
		Birch	4	0.0	0.0	NA
		Aspen	3	0.0	0.0	NA

[a] Sample size is repeated for each combination of functional group, successional stage, and forest type.
[b] Tukey HSD post-hoc tests were conducted after two-way ANOVA by permutation. Relevant ANOVA results are presented in Table A1.3. Different letters indicate a significant difference among forest cover types or successional stages for that functional group ($\alpha = 0.05$). NA, not available.

2.3.1.3 Taxa composition

Ordination of the bryophyte taxa for all forest types and all ages resulted in a two-dimensional NMDS solution that captured 39.6% of the variation in the original ranked distance matrix (Figure 2.3, stress 0.18). This level of stress indicates that the ordination is able to capture meaningful patterns in the data, with small distortions in the representation (McCune et al. 2002). The two axes from the NMDS represent two main ecological gradients: changes associated with stand age (NMDS 1) and leaf litter cover (NMDS 2; Table 2.4). There was a significant interaction between forest type and successional stage (interaction $F_{4,74} = 2.19$, $p = 0.003$; Table A1.2a).

During early succession (5-19 years since fire), taxa composition did not significantly differ among the three forest types ($F_{2,26} = 1.89$, $p = 0.057$; Table A1.2b). Stands of different types occupied a similar area of the multivariate space in the NMDS ordination (Figure 2.3a). Composition of bryophyte understory taxa in all forest types was dominated by colonizers such as *M. polymorpha*, *P. juniperinum*, *C. purpureus*, and taxa of the Bryaceae family (including taxa such as *Leptobryum pyriforme*, *Bryum* spp., and *Pohlia* spp.) (Figure 2.3a).

During mid-succession (20-60 years since fire), taxa composition started to diverge among forest types ($F_{2,39} = 2.848$, $p > 0.001$; Table A1.2c). Bryophyte communities in birch and aspen stands only partly overlapped the multivariate space occupied by communities in coniferous spruce stands (Figure 2.3b). Taxa composition in spruce stands differed from that of aspen stands, but was similar to that of birch stands, and there was no difference in taxa composition among the two deciduous forest types (*p* values in Table A1.4c). Rare taxa from the Hypnales order such as *Amblystegium* spp., *Drepanocladus* spp., *Brachythecium* spp., and *Ptilium crista-castrensis*, dominated the bryophyte layer in deciduous stands (Figure 2.3b). In spruce stands, feather mosses (*P. schreberi*, *H. splendens*) became dominant, along with *Dicranum* spp. and *Aulacomnium palustre*. Taxa composition in the earliest years of the mid-successional stage overlapped among all forest types.

Bryophyte communities in late succession (≥ 60 years since fire) were significantly different in taxa composition among forest types ($F_{2,9} = 4.429$, $p > 0.001$; Table A1.2d). Some bryophyte taxa found in older stands could not be identified, often because the specimens were too small to lead to a conclusive identification, but likely comprised the genera *Brachythecium*

and *Hypnum*, among others. Bryophyte taxa composition of aspen and birch stands was similar ($p = 0.417$; Table A1.4d), but differed from spruce stands ($p = 0.048$ and $p = 0.001$, respectively; Table A1.2d). In spruce stands, the feather mosses *H. splendens* and *P. schreberi* remained dominant components of the bryophyte carpet, and *Sphagnum* spp. cover increased.

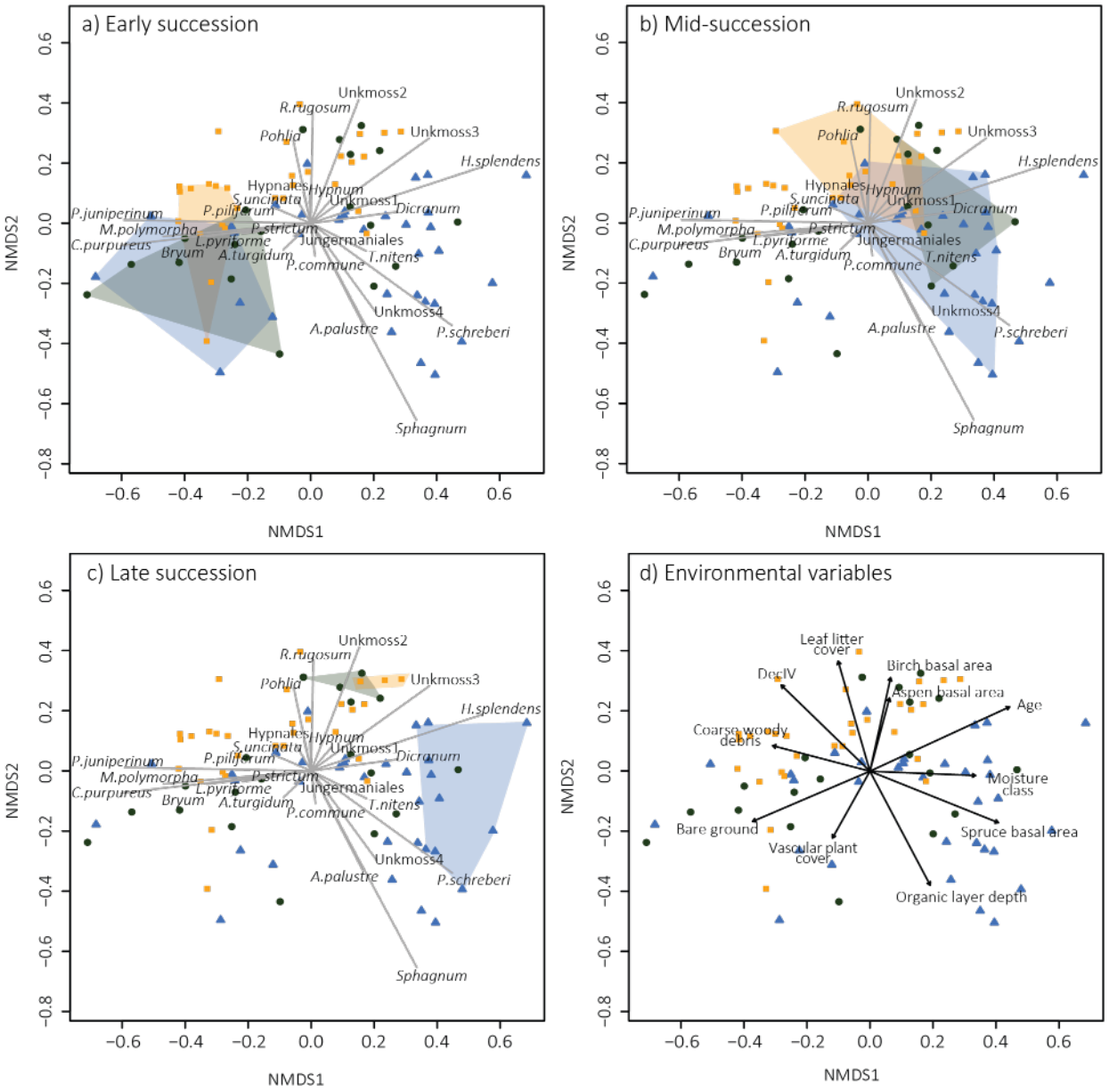

Figure 2.3: Distribution of the stands in a two-dimensional NMDS ordination (stress of 0.18, 100 iterations, Bray Curtis distance) based on bryophyte community composition (83 stands and 25 taxa). The two axes capture 39.6% of the variation in the original ranked matrix, with axes 1 and 2 capturing 33.1% and 6.5% of the variation, respectively. Individual points represent sample units (stands) grouped by forest type: spruce (blue, triangles), birch (green, circles) and aspen (yellow, squares). All stands are represented on each of the four panels, irrespective of age. Polygons in each panel are drawn to encompass stands representing (a) early succession (8-20 years since fire), (b) mid-succession (20-60 years since fire), and (c) late succession (more than 60 years since fire) (colour code same as stands); (d) black vectors show correlations with environmental and stand covariates.

Table 2.4: Axis loadings of environmental and stand covariates on the two NMDS axes.

	NMDS 1	**NMDS 2**	**r^2**	***p* value** [d]
Age (years)[a]	0.9085	0.4180	0.5290	0.001*
Bare ground cover (%)	-0.9195	-0.3930	0.3640	0.001*
Coarse woody debris cover (%)	-0.9674	0.2533	0.2265	0.001*
Leaf litter cover (%)	-0.2781	0.9606	0.2928	0.001*
DecIV index[b]	-0.7182	0.6959	0.3418	0.001*
Black spruce basal area[c]	0.9286	-0.3711	0.4283	0.001*
Aspen basal area[c]	0.2162	0.9763	0.2048	0.001*
Organic layer depth (cm)	0.4683	-0.8836	0.3708	0.001*
Moisture class (six classes)	0.9992	-0.0407	0.2505	0.001*
Vascular plant cover (%)	-0.4895	-0.8720	0.1311	0.005*
Birch basal area[c]	0.2595	0.9657	0.1281	0.009*
pH	-0.9967	-0.0812	0.0538	0.101
Elevation (m)	-0.7995	0.6007	0.0315	0.283
Lichen cover (%)	-0.1675	-0.9859	0.0236	0.374
Heatload	-0.9970	0.0781	0.0171	0.497
Shrub basal area[c]	-0.9999	-0.0125	0.0100	0.623

[a] Years since fire (age) based on sampling year minus year burned.
[b] Deciduous Importance Value index: see Alexander et al. (2012) for calculation.
[c] Total basal area calculated for each taxa group (cm^2 basal area per m^2 sample area). Tall shrubs included *Betula* spp., *Alnus* spp., and *Salix* spp.
[d] The r^2 values are Pearson correlation coefficients, and significance of the correlations was obtained from 999 permutations. An asterisk (*) indicates significant correlations at $\alpha = 0.05$.

2.3.2 Part II: environmental covariates related to bryophyte divergence

2.3.2.1 Total bryophyte cover

In the regression tree analysis, leaf litter was the best predictor of total bryophyte cover, with 76% cover identified as a threshold value (Figure 2.4). This threshold value was reached at about 20 years in aspen stands and at 40 years in birch stands, and only a few very young spruce stands presented such a high leaf litter cover, probably derived from the high cover of deciduous shrubs in those stands. When leaf litter cover was high, stands with more than 4% cover of bare ground had higher bryophyte cover (younger stands), while stands having less than 4% bare ground cover had lower bryophyte cover and corresponded to older stands (Figure 2.4). When leaf litter cover was low, depth of the organic layer was the next important variable associated with total bryophyte cover (Figure 2.4). Stands with an organic layer < 8 cm deep had a lower bryophyte cover and were slightly younger, and canopy composition was split evenly between spruce and deciduous stands. Stands with a deeper organic layer were mostly spruce stands and had the highest bryophyte cover of all groups (72%; Figure 2.4).

Leaf litter cover varied according to forest type and postfire successional stage ($F_{4,74} = 6.938$, $p < 0.0001$; Figure 2.5). During early succession, leaf litter cover was similar among forest types ($\bar{x} = 69\%$). Differences in leaf litter cover occurred during mid-succession when litter cover decreased to 50% in spruce stands and increased to about 76% (threshold identified by the regression tree) in both deciduous forest types. The difference in litter cover among stand types increased during the late successional stage, with litter cover reduced to a mean of 33% in spruce stands, and increased to 94% in birch and aspen stands. The divergence in leaf litter cover among the forest types occurred at 20-40 years after fire.

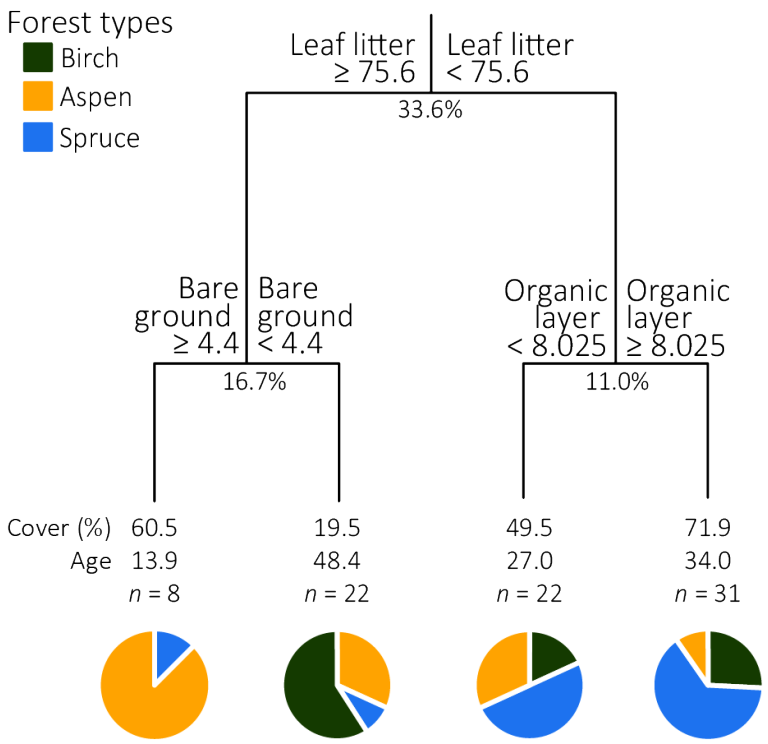

Figure 2.4: Univariate regression tree partitioning variation in total bryophyte cover (%). Thresholds associated with environmental variables for each split are shown next to each node of the tree, along with the percentage of total variation explained by each split. The percentage of the variation explained by the tree is 61.2% and the cross-validated error to estimate prediction error on new samples is 74.6%. Each terminal group is shown with its average bryophyte cover (%), age (years since fire), and number of stands; pie charts show the relative abundance of forest types in each group.

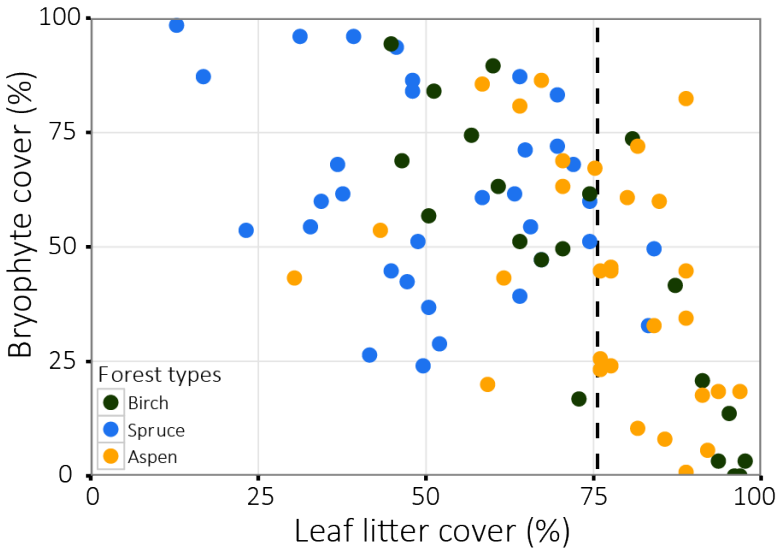

Figure 2.5: Variations in total bryophyte cover (%) versus leaf litter cover (%) in stands (n=83) dominated by spruce, birch, or aspen. The dashed vertical line identifies the 75.6% threshold in leaf litter cover identified by the regression tree analysis.

2.3.2.2 Functional groups and taxa composition

Multivariate regression trees predicting functional group cover or bryophyte taxa composition were very similar (Figure 2.6). In both cases, bare ground was the most prominent covariate associated with bryophyte communities, as it captured a threshold between younger (high bare ground cover) and older (low bare ground cover) stands. Leaf litter cover was the next most important variable, with thresholds of 78.8% for functional groups and 76.8% for taxa composition (Figure 2.6). A lower leaf litter cover was associated with either younger stands or mature spruce stands for functional groups (Figure 2.6a), or with mature spruce stands for the taxa composition data (Figure 2.6b). In both regression trees, stands with a high leaf litter cover were associated with distinct bryophyte communities mostly in mature deciduous stands (Figure 2.6).

Results from the environmental vector fitting on the NMDS indicated that bare ground cover and age, which were inversely correlated to each other, were the main variables associated with the distribution of the stands along axis 1 in the multidimensional space of the ordination (Table 2.4). Leaf litter cover was the main variable associated with the distribution of stands along axis 2 (Figure 2.3d, Table 2.4). Spruce stands in mid- and late succession were associated with a thick organic layer, higher spruce basal area, and higher soil moisture. Birch and aspen stands in mid- and late succession were associated with higher basal areas of birch and aspen and higher leaf litter cover (Figure 2.3d; Table 2.4). We found a significant interaction of forest type and successional stage ($F_{4,74} = 2.194$, $p = 0.0030$; Table A1.4a) in explaining taxa composition, which supported the visual interpretation of the NMDS plot in Figures 2.3a-c.

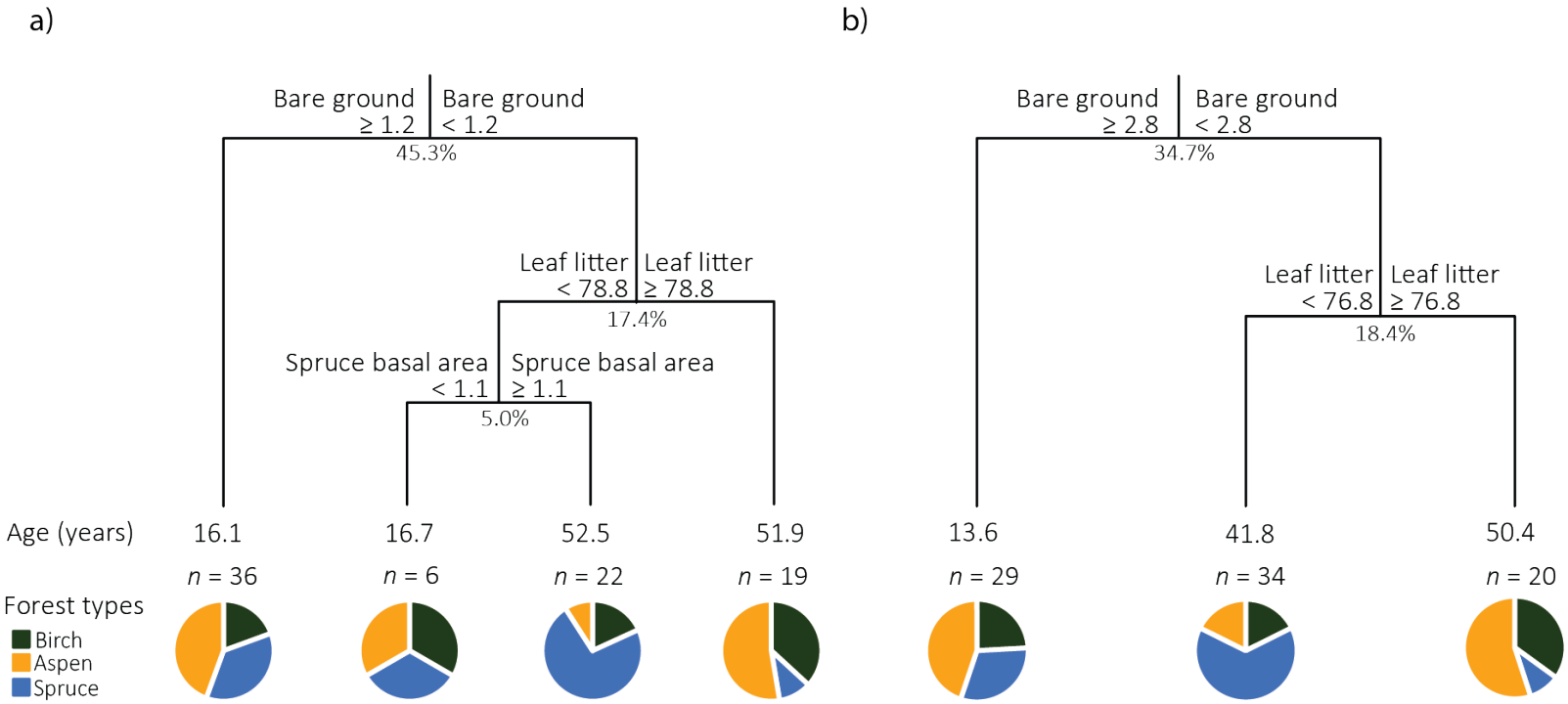

Figure 2.6: Multivariate regression trees partitioning the Bray Curtis distances among stands (n = 83) calculated from (a) functional group cover (n = 5) and (b) bryophyte taxa composition (n = 25). Thresholds associated with environmental variables for each split are shown next to each node of the tree, along with the percentage of total variation explained by each split. In (a), the percentage of variation explained by the tree is 64.8% and the cross-validated error is 52.5%; in (b), the percentage of variation explained by the tree in is 43.5%, the error is 56.5%, and the cross-validated error is 67.1%. Each terminal group is shown with its average bryophyte cover (%), age (years since fire), and number of stands; pie charts show the relative abundance of forest types in each group.

2.4 Discussion

We identified a clear time scale of changes in bryophyte abundance and taxa composition driven by canopy composition in boreal forests of interior Alaska. Our results are based on a unique empirical dataset documenting patterns of bryophyte cover and community composition across an 8 to 163 year chronosequence of contrasting boreal forest canopy types. To our knowledge, few studies have focused on boreal understory communities in different forest types of similar ages (Hart and Chen 2006) or included different successional stages in such a comparison. Our findings support the interpretation of bryophyte communities diverging in response to changing canopy composition (De Grandpré et al. 1993, Hart and Chen 2006). Bryophyte cover and community composition were similar among forest types in early succession but then diverged between coniferous (black spruce) and deciduous (Alaska paper birch and trembling aspen) forest types between 20 and 40 years after fire. These changes in the bryophyte understory appear to be driven by differences in litter cover caused by higher annual production of litter in deciduous stands (Melvin et al. 2015), consistent with the hypothesis that chemical and physical aspects of leaf litter affect the growth and survival of understory taxa (Barbier et al. 2008). A threshold of about 75% leaf litter cover was associated with a large reduction in bryophyte cover and a change in taxa composition among the 83 stands in our study. Our results identify unique successional patterns strongly related to canopy types and highlight the important role of leaf litter in regulating bryophyte communities.

Bryophyte communities in postfire aspen, birch, and spruce forests were similar in the early successional stages during the first 6-20 years after fire. Thus, we must reject the hypothesis that differences in bryophyte composition among mature coniferous vs. deciduous stands are attributable to distinct successional trajectories that arise immediately after fire. Instead, our data suggest that bryophyte communities in early succession are strongly influenced by common dynamics of colonization that are similar regardless of the composition of the young forest canopy. Young stands of all forest types had a high bryophyte cover (~75%) dominated by colonizing and pyrophilic taxa such as *Ceratodon purpureus, Leptobryum pyriforme, Marchantia polymorpha,* and *Polytrichum* spp. (Hart and Chen 2006, Turetsky et al. 2010). Early successional stands had more exposed bare ground than older stands, which is representative of the time necessary to recruit bryophytes from diaspores. The youngest stands were dominated by

C. purpureus (6-20 years), a pioneer taxa intolerant of competition, which was likely overgrown by the larger and more competitive *P. juniperinum* and *P. commune* that dominated stands 20-40 years after fire (Gloaguen 1990). Extensive carpets of *Polytrichum* spp. are common following disturbances in many ecosystems (Foster 1985, Gloaguen 1990).

The similarity among bryophyte communities that we observed in early succession contrasts with existing research showing impacts of fire severity on bryophyte abundance and richness two years after fire (Bernhardt et al. 2011, Hollingsworth et al. 2013). We hypothesize that the short-term responses of bryophyte communities to fire severity are likely masked within a few years by the opposing impacts of fire severity on survival versus colonization. Immediately after fire, incomplete combustion of the organic layer in low severity fires promotes bryophyte abundance because of remnant live bryophytes and fragments (Thomas et al. 1994, Bernhardt et al. 2011, Fenton and Bergeron 2013). However, high-severity fires provide seedbeds of mineral soil and ashes that are good substrates for subsequent bryophyte colonization (Thomas et al. 1994, Bernhardt et al. 2011, Fenton and Bergeron 2013). Thus, bryophyte regeneration after fire is supported by opposite mechanisms of recruitment depending on the fire behaviour and leads to a similar trajectory of bryophyte development in early successional stands.

At the transition between early and mid-successional stages, the cover of colonizing taxa declined abruptly and at a similar pace in all forest types. We suspect that different processes led to the change in bryophyte communities in coniferous vs. deciduous forest types. In spruce stands, the decrease in abundance of colonizers between 20 and 40 years since fire is synchronous with an increase in abundance of pleurocarpous feather mosses, suggesting that competition between colonizing taxa and feather mosses may be an important process (autogenic succession) (Rydin 1997). In deciduous stands, this decline in colonizers corresponded to a significant increase in leaf litter cover, meaning that in deciduous stands, external factors such as leaf litter and canopy closure may be more important (allogenic succession). More detailed studies, e.g. manipulative experiments with reciprocal bryophyte transplants, would help to clarify the role of competition and environmental variables in bryophyte succession.

By 40 years after fire, bryophyte cover and community composition differed among forest cover types, indicating a divergence in successional pathways related to canopy cover in mid-succession. This time period corresponds with the timing of canopy closure in spruce-

dominated stands (Chapin et al. 2006a), which comes with a decrease in light availability and, as we observed, a decrease in deciduous tree and shrub litter in spruce stands (Turetsky et al. 2010). Bryophyte cover was high in mid- and late successional spruce stands (close to 100% in older stands) and was dominated by the feather mosses *Pleurozium schreberi* and *Hylocomium splendens*. *Pleurozium schreberi* remained the dominant taxa throughout most of this period, always covering between 5% and 15% more than *H. splendens* until about 100 years after fire. In some of our oldest black spruce stands, S*phagnum* spp. was present. The few stands older than 90 years limit the conclusions that we can draw for this successional stage. The patterns that we observed are consistent with other studies of black spruce forests that document establishment of *Sphagnum* after about 100 years after fire, following changes in soil organic layer depth, temperature, and moisture initiated by extensive development of feather mosses (Fenton and Bergeron 2006, Turetsky et al. 2010). Bryophyte succession in spruce stands seems first to be triggered by the decrease in deciduous leaf litter associated with spruce canopy closure and afterwards to be driven by autogenic processes influenced by feather mosses.

Canopy composition and associated variations in leaf litter production are recognized as major factors influencing understory communities in boreal forests (De Grandpré et al. 1993, Hart and Chen 2006). Broadleaf litter may form a physical barrier to bryophyte growth (Van Cleve et al. 1983b, Startsev et al. 2008), increase nutrient availability in ways that reduce the competitive ability of bryophytes, and have allelopathic effects (Startsev et al. 2008). Strong (2011) found a decline in the abundance of *Hylocomium splendens* in northwestern Canada in stands with aspen canopy cover over 40%, consistent with our observations of a significant decline in bryophyte abundance above a threshold of 75% leaf litter cover, or roughly 50% deciduous canopy cover (estimated from basal area). Bryophytes in deciduous stands were rare throughout mid- and late succession and often restricted to decomposing logs or mounds where leaf litter was blown away. Increases in canopy gaps and decomposing logs that shed leaf litter with aging deciduous stands may allow feather mosses to increase in abundance late in succession (Jonsson and Esseen 1990). This pattern was reported for aspen stands > 75 years old by Strong (2009) and was apparent in one of our old aspen stands (Figure 2.1); however, our inference is constrained by a low sample size of deciduous-dominated forest stands older than 100 years. Nevertheless, the change in bryophyte communities in deciduous stands seems to be mostly driven by allogenic factors linked to canopy development.

The dominance of pleurocarpous feather mosses in spruce stands (Turetsky et al. 2012) and their low abundance in aspen and birch stands are critical from an ecosystem functioning perspective. Feather mosses are long-lived perennials, relatively large and fast growing, and are good competitors that can quickly take advantage of newly opened substrates by shoot encroachment (Frego 1996). Feather mosses build organic layers that enhance soil insulation and maintain shallow active layers (Turetsky et al. 2012), as well as retain soil moisture (Turetsky et al. 2010). We found a positive association between bryophyte abundance and organic layer depth, which can be linked to re-establishment of permafrost 20-30 years after fire (Viereck et al. 2008). Associations between feather mosses and cyanobacteria are also involved in biological dinitrogen (N_2) fixation (DeLuca et al. 2002). If deciduous seedlings take advantage of exposed mineral soil following severe fires and replace black spruce as the dominant forest canopy (Johnstone et al. 2004, 2010b), increased production of leaf litter may prevent the subsequent recovery of feather mosses during succession. Low bryophyte cover in deciduous stands in mid-succession promotes the stability of this alternate forest type by supporting warm and dry soil conditions with high nutrient availability (Johnstone et al. 2010a). Colonizing bryophyte taxa such as *Polytrichum* spp. do not have the same impacts on the ecosystem in terms of insulation (Soudzilovskaia et al. 2011), water retention (Elumeeva et al. 2011), and hosting N fixers (Gavazov et al. 2010).

Divergence of bryophyte communities among canopy types at about 40 years after fire, i.e. during mid-succession, indicated that divergence in succession is arising due to increasing canopy effects as stands mature, rather than differences being caused by direct initial effects of fire severity. Black spruce forests are the most widespread forest type in interior Alaska (Calef et al. 2005), and bryophyte functional traits promote the resilience of black spruce forests through their effects on soil moisture, soil temperature, nutrient cycling, and flammability, among others (Johnstone et al. 2010a, Turetsky et al. 2012). Feedbacks between canopy, leaf litter production, and understory bryophytes throughout postfire forest regeneration are keys to our understanding of ecosystem resilience in interior Alaska in a context of climate change (Johnstone et al. 2010a). Changes in canopy dominance towards more mixed and deciduous stands will therefore have major impacts on boreal ecosystem functioning through the negative impacts of deciduous trees and leaf litter on feather mosses.

2.5 Conclusion

Bryophyte communities in interior Alaska followed divergent successional trajectories associated with vascular canopy cover (deciduous vs. coniferous) despite similar patterns during the first 40 years after fire. Autogenic processes such as competition within the bryophyte layer in spruce stands and allogenic processes such as changes in leaf litter cover in deciduous stands were associated with divergence in bryophyte communities among forest types, with a transition to feather moss in spruce stands and low bryophyte abundance in birch and aspen stands. Black spruce stands in mid- and late succession are characterized by an understory of feather mosses and *Sphagnum* spp. with thick organic layers, conditions that support further dominance of black spruce (Johnstone et al. 2010a). However, in deciduous stands, reduction in bryophyte cover at a threshold of ~75% leaf litter cover supports conditions favourable to maintenance of deciduous dominance such as warm soils and high nutrient availability. Feather mosses also affect important ecosystem processes in boreal ecosystems such as carbon storage, soil microclimate regulation, and N_2-fixation. Changes in fire regime that lead to an increase in deciduousness in interior Alaska (Mann et al. 2012) will likely also alter bryophyte communities and have cascading impacts on boreal ecosystem functioning. Temporal changes in bryophyte communities induced by leaf litter cover is a key aspect to our understanding of the processes that stabilize compositional patterns of boreal forests and is critical for predicting ecosystem responses to environmental change.

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CHAPTER 3: LEAF LITTER IMPACTS ON FEATHER MOSS IN DECIDUOUS AND CONIFEROUS BOREAL FORESTS

3.1 Introduction

Much attention has recently been paid to the complex biological processes that underlie the patterns of stable biological communities in natural systems. Plant-soil-microbe feedbacks, which occur when plants affect soils through physical, chemical, or biological processes in ways that in turn affect plant communities, influence the ecological resilience of terrestrial ecosystems and their effects range in scale from microbial communities to entire ecosystems (Gunderson 2000, Ehrenfeld et al. 2005, Chapin et al. 2010). Ground covering bryophytes are at the interface between above and belowground processes and can therefore play major roles in plant-soil feedbacks (Lindo et al. 2013). Bryophytes often carry out ecosystem processes of an importance disproportionate to their contribution to stand biomass (Sveinbjörnsson and Oechel 1992), and some bryophyte groups such as feather mosses and Sphagna are considered as keystone species in boreal ecosystems (Rochefort 2000, Frego 2007, Turetsky et al. 2010, Turetsky et al. 2012). Bryophytes produce low bulk density and recalcitrant (slow decomposing) litter which leads to the accumulation of thick organic layers and promote cold, wet, and nutrient-poor soils, which in turn limits vascular plant productivity (e.g., Chapin et al. 2010, Turetsky et al. 2010). Bryophytes can also host dinitrogen (N_2)-fixing cyanobacteria which may provide important sources of N in nutrient poor ecosystems (DeLuca et al. 2002, DeLuca et al. 2007). Plant-soil feedbacks are a likely mechanism regulating ecosystem resilience, and bryophyte-mediated feedbacks are very likely in high latitude ecosystems where they are abundant (Turestky 2003, Turetsky et al. 2012).

Bryophytes create plant-soil feedbacks that lead to the resilience of coniferous forests (Chapin et al. 2010, Johnstone et al. 2010a). Ecological resilience is defined as the capacity of a system to tolerate disturbance of a certain intensity without altering its fundamental feedbacks, processes, and population structures (Holling 1973, Gunderson 2000). In interior Alaska, two major stable forest types coexist: broadleaf deciduous stands with low moss cover dominated by aspen (*Populus tremuloides* Michx.) or birch (*Betula neoalaskana* Sarg.) and coniferous stands with a high moss cover dominated by black spruce (*Picea mariana* [Mill.] B.S.P.) (Chapin et al. 2006a). Following fire, the two major stand types typically self-regenerate, indicating highly resilient deciduous and coniferous stands (Chapin et al. 2006a, Chapin et al. 2006b, Johnstone et al. 2010a). Resilience of both deciduous and coniferous stands is affected by their specific feedbacks, the physical environment (especially soil organic matter) and plant traits (e.g., litter

inputs), as well as the characteristics of the disturbance that disrupts the community (Chapin et al. 2010, Johnstone et al. 2010). In black spruce stands, the shaded, cool and moist forest floors and low litter fall promote the establishment of feather mosses, which favour slow nutrient turnover, slow decomposition, and conservative growth strategies (Hollingsworth et al. 2006, Johnstone et al. 2010a). Such conditions further promote moss productivity, accumulation of thick organic layers, and dominance of black spruce (Van Cleve et al. 1983a, Foster 1985, Fenton et al. 2005). Soils in deciduous stands are characterized by shallow organic layers, deep active layers above permafrost, warm temperatures, good drainage, and fast decomposition and nutrient turnover rates, conditions that are detrimental to moss growth (Hart and Chen 2006, Johnstone et al. 2010a, Turetsky et al. 2010). Furthermore, the high input rate of leaf litter in deciduous stands has been hypothesized as the main factor limiting moss establishment and productivity, as prostrate mosses tend to get buried (Sveinbjörnsson and Oechel 1992, Johnstone et al. 2010a). Because of their key role in stabilizing plant-soil feedbacks, the presence or absence of bryophytes, and their cover and species composition, could provide key controls over succession and regeneration of boreal forests (Johnstone et al. 2010a, Turetsky et al. 2012).

In boreal forests, interactions between fire and moss accumulation on the forest floor are key in shaping post-fire regeneration and support or disrupt stable patterns of forest succession. Contemporary increases in fire severity in Alaska and northwestern Canada, and therefore deeper combustion of the moss and organic soil layers, have caused shifts in canopy dominance after fire from black spruce to broadleaf deciduous trees, disrupting historical patterns of forest regeneration (Johnstone et al. 2010a, Johnstone et al. 2010b). In black spruce stands, thick organic moss layers retain moisture and therefore limit the potential for deep smoldering combustion of the organic layer during fires (Viereck et al. 1983, Miyanishi and Johnson 2002, Chapin et al. 2004). Post-fire burned organic matter is a poor quality seedbed (Dyrness and Norum 1983, Johnstone and Chapin 2006, Greene et al. 2007), but black spruce can overcome this constraint through its large seedbanks in semiserotinous cones (Zasada et al. 1992, Johnstone and Chapin 2006, Greene et al. 2007). Alternatively, mineral soils exposed following severe fires that burned through the organic layer are high quality seedbeds. Thus, fast-growing deciduous species that possess small wind-dispersed seeds are able to establish and then have a competitive advantage over black spruce (Johnstone and Chapin 2006, Greene et al. 2007, Johnstone et al. 2010b). Deciduous stands do not develop substantial moss layers (e.g., Chapter 2), therefore such

changes in forest composition and structure will lead to a switch in the plant-soil feedbacks from a moss litter and thick organic layer domain to a deciduous broadleaf litter and shallow organic layer domain (Johnstone et al. 2010b, Turetsky et al. 2010).

The cause of the discrepancy in moss abundance between decidous broadleaf and coniferous stands has been attributed to different environmental conditions specific to each forest type, such as colder and moister conditions in spruce stands, or to higher leaf litter inputs in deciduous stands (e.g., Van Cleve et al. 1983b, also reviewed in Chapter 2). We currently do not have a good understanding, or quantitative estimates, of the relative importance of environmental conditions and leaf litter in driving differences in moss growth and biomass accumulation. Leaf litter can have physical impacts on mosses by its crushing effect, barrier to establishment and vertical growth, as well as light interception (Van Cleve et al. 1983b, Beatty and Sholes 1988, Startsev et al. 2008) because the prostrate form and slow growth rate of mosses make them vulnerable to burying (Ewald 2000, Barbier et al. 2008). Deciduous leaf litter is generally higher in base cations and pH than coniferous needles (Paré and Bergeron 1996), which increases nutrient availability in ways that may reduce the competitive ability of moss compared to vascular plants (Messier et al. 1998, Côté et al. 2000, Turetsky et al. 2012). Deciduous trees also contain phenols in their leaves, which may have allelopathic effects on moss (Légaré et al. 2005, Startsev et al. 2008). Reduced moss cover in deciduous stands leads to thin, warm and dry organic layers that further promotes deciduous dominance (Chapin et al. 2006b, Johnstone et al. 2010a, Johnstone et al. 2010b). Thus, teasing out the mechanisms that drive patterns of moss abundance and growth under deciduous and coniferous stands is necessary to understand a major stabilizing plant-soil feedbacks of boreal forests.

In this study, I used field-based experiments in order to 1) tease apart the respective impacts of forest type and leaf litter on moss growth, health (greenness), architecture (morphology), and biomass accumulation, and 2) separate the relative impacts of physical and chemical effects of leaf litter on moss growth. An experimental approach using feather moss transplants in black spruce and Alaska paper birch stands, combined with leaf litter treatments, allowed me to separate canopy induced feedbacks (deciduous leaf litter) from other environmental drivers (e.g. moisture or pH). The experiment tests the following predictions and associated hypotheses about key mechanisms that may regulate patterns of moss abundance among forest stand types. 1) If leaf litter is the main mechanism limiting moss abundance in

deciduous stands, then mosses subjected to leaf litter additions should present lower growth and health in both stand types. 2) In contrast, if environmental conditions are the primary mechanism making spruce stands more favourable for moss growth and health than birch stands, then mosses should grow better in spruce stands regardless of leaf litter inputs. A parallel experiment manipulating the form of leaf litter applied to mosses was aimed at testing the specific mechanisms that may drive the impacts of leaf litter on mosses. I predicted that a) if the physical effect of leaf litter is a significant mechanism affecting moss growth, then mosses treated with only weight and shading should grow less; b) if chemicals leaching out of the leaves (e.g. phenols) are important, then mosses subjected to application of leaf leachates should grow less; or c) if both the physical and chemical aspects of leaf litter are important, then additive or multiplicative effects could be observed with natural leaf litter application on the mosses. Together, these tests of the mechanisms that potentially drive patterns of moss abundance in boreal forest stands fill important gaps in our understanding of how changes in forest types may be stabilized by plant-soil feedback cycles associated with moss abundance in the boreal forest.

3.2 Methods

3.2.1 Study area

My sites are situated in the Tanana Valley State Forest near Fairbanks, Alaska, (64° 53' N, 148° 23' W), and are part of the Regional Site Network of the Bonanza Creek Long-Term Ecological Research program (www.lter.uaf.edu/). In interior Alaska, the mean annual air temperature is -2.5°C and the growing season lasts about 135 days (Hinzman et al. 2005). About 35% of the annual 286 mm of precipitation falls as snow (Hinzman et al. 2005). In 2012, I established my experiments in three pairs (blocks) of adjacent black spruce and Alaska paper birch stands (see Melvin et al. (2015) for a detailed description of the stands). All stands originated from a 1958 fire, and all birch stands had evidence of black spruce presence prior to the last fire. Near-surface permafrost was present in the spruce stands, but not in birch stands. Two of the blocks (A and B) were on north-facing slopes and representative of upland forests in the area. Block C was on a shallower slope adjacent to a creek, and the plant community was more riparian. While ground cover in spruce stands was dominated by the feather mosses *Hylocomium splendens* (Hewd) Schimp. and *Pleurozium schreberi* (Brid.) Mitt., ground cover in

birch stands was mostly composed of birch leaf litter (Melvin et al. 2015). Five plots (10 m x 10 m) were randomly established in each stand (15 plots dominated by each forest type, 30 plots total).

3.2.2 Moss transplant experiment

The experiment ran from June 2012 to August 2015, with initial set up and field measurements conducted by a team of research collaborators and field assistants. Within each of the 15 black spruce plots distributed among the 3 experimental blocks, we excavated six cores of live *H. splendens* dominated moss carpet (30.48 cm in diameter) down to the fibric horizon of the organic layer. Once the moss cores from one black spruce stand were collected, they were randomly assigned to be transplanted to either the birch or spruce stand in the same block. In the spruce stands, mosses were randomly transplanted into 3 out of the 6 harvested locations and in the birch stands, we removed the forest floor to the mineral soil at a randomly chosen location before installing the moss transplant. We identified procedural control areas where *H. splendens* was naturally abundant in each plot as a fourth treatment to assess any effects of the transplanting procedure on moss growth. In some of the birch stands, these areas were small or found on decomposing logs. The 90 moss transplants and 30 procedural control moss areas will hereafter be referred to as sampling units (SU).

All moss transplants were randomly assigned to one of three treatments: litter exclusion, litter addition, and ambient litter (Figure 3.1). In the ambient litter treatment, the transplants received natural litter fall. In the litter exclusion and addition treatments, all natural deciduous leaf litter was prevented from falling on to the moss transplants using plastic mesh tents that were installed in late August and removed after the birch trees had lost their leaves in early October. In the litter addition treatment, birch litter was manually added to transplants at the ambient input rate each year in early October. Annual birch litter production was assessed using three randomly placed litter collection baskets (62.2 cm x 45.4 cm, lined with fiberglass window screen) within each birch plot. Litter was collected from the baskets following leaf fall in September and October 2012-2014 in order to calculate the ambient input rate. For consistency, the 2012 leaf litter input rate (224.8 g dry leaf litter m^{-2} $year^{-1}$) was used every year for my leaf litter additions. Starting in October 2013, a loose net was installed over the addition treatments to ensure that the litter would stay in place over the fall and winter. These nets were removed during the growing season. Leaf litter cover of all SUs was monitored using pictures taken in

June and October every year, and analyzed with Adobe Photoshop CS5 (Adobe Systems, Mountain View, CA). This allowed us to consider litter as both a categorical (treatment) and continuous (percent cover) variable.

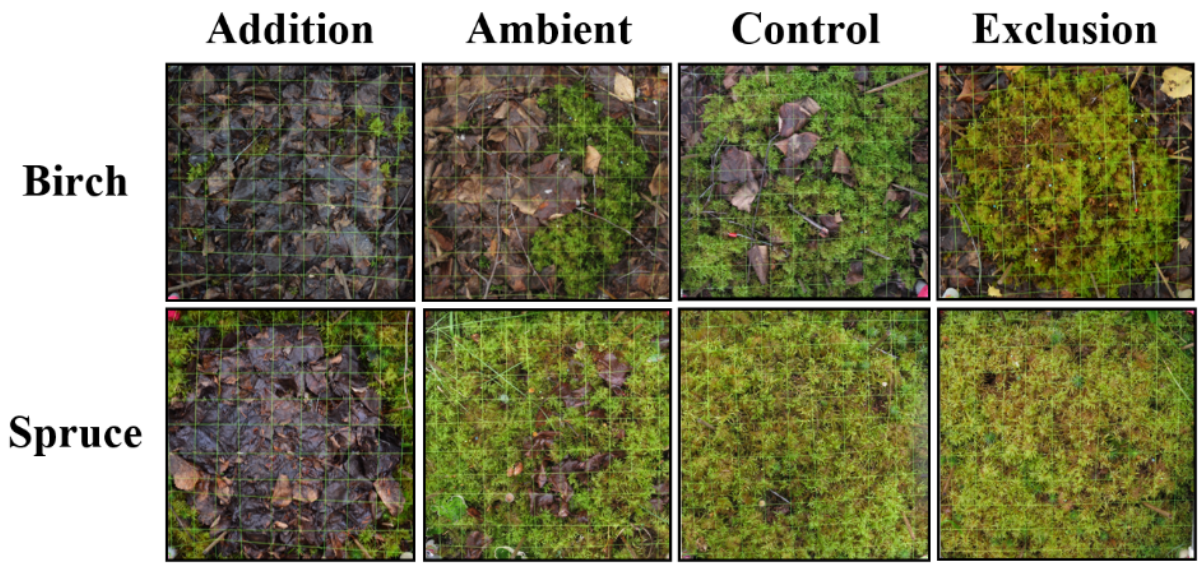

Figure 3.1: Photographs of sampling units (SU) and leaf litter treatments in spruce and birch stands. Addition, ambient and exclusions are transplants, while controls represent a procedural control established using naturally occurring patches of moss. The fine grid in the photographs is the quadrat strung with fishing line (3 cm apart) used to locate moss individuals. Pictures by M. Jean.

3.2.3 Leachates experiment

In 2014, I initiated a second experiment to separate the mechanical and chemical effects of birch leaf litter on moss growing in the black spruce stand of block A. Five plots (2 x 2 m) were randomly located in the vicinity of the five plots used for the transplant experiment. Four subplots (50 x 50 cm) were placed within each plot and randomly assigned to one of the following treatments: leached leaves, leaf leachates, fresh leaf litter and control. Treatment assignments were constrained to always keep the leachate treatment on the downslope side of the plot. The litter input rate for all treatments was set at the measured rate of birch leaf fall (leaf mass per unit area) in 2012 and was conducted the same way as in the transplant experiment. The leached leaves treatment consisted of adding birch leaves that had been leached in deionized water at room temperature (~20 °C) for 7 days (Nilsson and Zackrisson 1992) to remove water-soluble, leachable components from the leaves but keep their physical structure intact. We changed the water three times a day and manually stirred both before and after each change. The leaves were allowed to dry at room temperature for 24 hours before being applied to the subplots. To create the leaf leachates treatment, we leached birch leaves (total of 281 g dry leaf litter for the 5 subplots) into 5 L of rain water for 24 hours at room temperature, while manually stirring the leaf litter for 2 minutes every 4 hours. This ratio of water to leaves was selected in order to submerse all leaves, and is similar to what has been done in other leaching experiments (Nilsson and Zackrisson 1992). We filtered the solution using filter paper (Reeve angel grade 202 filter paper, catalog no 5202-090) and obtained ~4 L of leachates. We then applied 800 mL of leachates to the leachate treatment subplots at three times (September 2014, June 2015, and August 2015), and applied 800 mL of rainwater to the other three treatments at the same times.

3.2.4 Environmental conditions and leaf litter inputs

In June 2012, one meteorological station (Onset, Bourne MA) was installed in each stand (n = 6). At each station, air temperature, relative humidity, photosynthetically active radiation, soil temperature (10 cm from the organic layer surface) and soil moisture (10 cm from the mineral soil surface) were measured hourly. The forest floor community was characterized using the point-intercept sampling at 10 random locations within each plot during the summer of 2012 (Melvin et al. 2015). A 0.5 m x 0.5 m frame with 16 intersections within the grid was used. At each intersection, hits on moss, herbs, lichen, shrubs, leaf litter, and "other" plants were recorded. Organic layer depth, N mineralization and nitrification rates, and pH were measured

using soil cores collected in 2013 in each plot (Melvin et al. 2015). Canopy cover was measured with hemispherical pictures taken 30 cm above the surface of each SU and processed with Gap Light Analyzer (Frazer et al. 1999). Site characteristics are summarized in Table A5.1.

3.2.5 Moss measurements

3.2.5.1 Moss greenness

Plant greenness is an indicator of potential growth, water content, and chlorophyll or N content, which are indicators of plant health (Karcher and Richardson 2003, Olthof and Latifovic 2007, Malenovsky et al. 2013). To quantify moss colour, I took pictures of all the SUs without leaf litter in September 2012 and August 2015 using digital cameras, and calculated the average hue, saturation and brightness in Adobe Photoshop CS5 (Adobe Systems, Mountain View, CA). These values were used to calculate a Dark Green Colour Index (DGCI; Karcher and Richardson 2003). As mosses are typically not as deep green as vascular plants, I used a modified DGCI index by lowering the optimal hue of green. A value of 1 corresponded to a very green moss, and a value of 0 was not green. Not all pictures were taken with the same camera models, or under a similar lighting. However, I make the assumption that discrepancies in baseline light levels of camera are accounted for by the inclusion of the random effects of block, forest and plots, since their sampling was nested in space and time. A few other methods to measure moss colour were attempted and their outcomes are presented in Appendix 2.

3.2.5.2 Marked individuals, biomass and allometry

In this study, I focused on the growth on the two dominant feather mosses at my sites: *H. splendens* and *P. schreberi*. *Hylocomium splendens* has a strong apical dominance and a modular growth form, creating a new 'step' (hereafter called segment) every year, which facilitate monitoring and measurement of moss growth (Tamm 1953, Busby et al. 1978, Økland 1995). The production of new segments occurs by branching, i.e. the new segment grows on the segment of the previous year. It takes about 1.5 years for a segment to be considered mature and to have reached its full growth in length and width (Økland 1995). After about 3-4 years, the shoots become buried, photosynthesis stops, and decomposition begins (Økland 1995). *Pleurozium schreberi* grows continuously in length, but also in width, adding new branches along the stem and elongating branches from previous years (Longton and Greene 1969, Benscoter and Vitt 2007). Growth of multiple segments in one year, or growth starting on an

older shoot may occur, but were found to be rare in Sweden and Norway (Tamm 1953, Økland 1995).

Good estimates of moss growth and contribution to annual net primary production (ANPP) rely on accurate measurements of annual moss growth (Benscoter and Vitt 2007). This can be challenging when trying to conduct repeated measures on feather mosses, as typical measurements methods using vertical growth such as cranked wires (Clymo 1970) or marking of the stem (Camill et al. 2001) do not account for lateral growth (Benscoter and Vitt 2007), or involve destructive sampling over a netting (Bond-Lamberty et al. 2004). We tried three different methods to measure moss growth: individual measurements, height of the SU from the forest floor and a cranked wire transect (Appendix 2). In this chapter, I used a method that was developed specifically for *H. splendens* by Økland (1995). Slit PVC rings of different colours (HAMA plastic beads, Malte Haaning Plastics Co., Denmark; outer diameter 2.5 mm and inner diameter 1 mm) were placed as markers at the base of moss segments using tweezers (Økland 1995), which allowed me to identify the approximate age of each segment (Figure 3.2).

I marked five randomly selected *H. splendens* shoots in the 120 SUs of the moss transplant experiment (n = 600) in June 2013 and an additional five random shoots in September 2013 (total n = 1200). Ten individuals of *H. splendens* and five of *P. schreberi* were tagged in each subplot of the leachates experiment in September 2014 and June 2015, respectively. The location of all mosses was recorded using a grid system, which allowed me to re-measure the same individuals over time. I measured the marked individuals in June and September 2013, June and September 2014, and June and August 2015 for the transplant experiment, and in September 2014 and 2015 for the leachates experiment. In 2013 and 2014, mosses that were broken by experimenters or could not be relocated (~3% of moss individuals per sampling period) were replaced in order to maintain my sample size.

For each marked moss individual, I measured the length and width of each segment to the nearest millimeter. I also recorded buds (< 1 x 1 mm), sporophytes, and occurrences of multiple branching. When multiple branches occurred, their length and width were also measured according to their equivalent segment on the dominant moss axis. Length from the bead to the tip of the moss and width at the largest point were taken for each *P. schreberi* shoot. All shoot were destructively harvested in August 2015 and the final sample weights, dimensions, and

sporophyte counts were recorded in the laboratory. To verify that there were no initial differences in moss size, I harvested, measured and weighed the moss segment below the blue bead (Figure 3.2), as this segment should have matured in 2012, i.e. before experimental manipulations of leaf litter.

In September 2013, June 2014, and September 2014, I collected a core of *H. splendens* (100 cm^2) in the proximity of each plot, which allowed calculation of moss shoot density. I used 5 to 20 moss samples from each core to build allometric equations between *H. splendens* length, width, and weight (Appendix 3). These equations were used to estimate biomass of the tagged *H. splendens* at each measurement period using their length, width and segment position along the moss shoot. Estimates of biomass for *P. schreberi* in June 2015 were done by creating simple allometric equations with the width and length from the destructive harvest of August 2015. I created four response variables using this complete dataset: total biomass and number of segments per moss shoot, and biomass and number of segments for the dominant axis of the moss sample. I used a distinction between total moss and the dominant axis to account for a possible lower probability of detection of side branches in the field, compared to my final laboratory measurements. The results for the total and dominant moss axis variables were very similar (Appendix 2), and I therefore only present the results from total moss biomass and number of segments. Lastly, I counted the number of side-segments produced per shoot as the difference between the total number of segments and the number of segments on the dominant chain. All moss variables from the transplant experiment were averaged per transplant, as we had between 4 and 10 shoots per SU at the end of the experiment, and some individuals were lost over time and new ones were added.

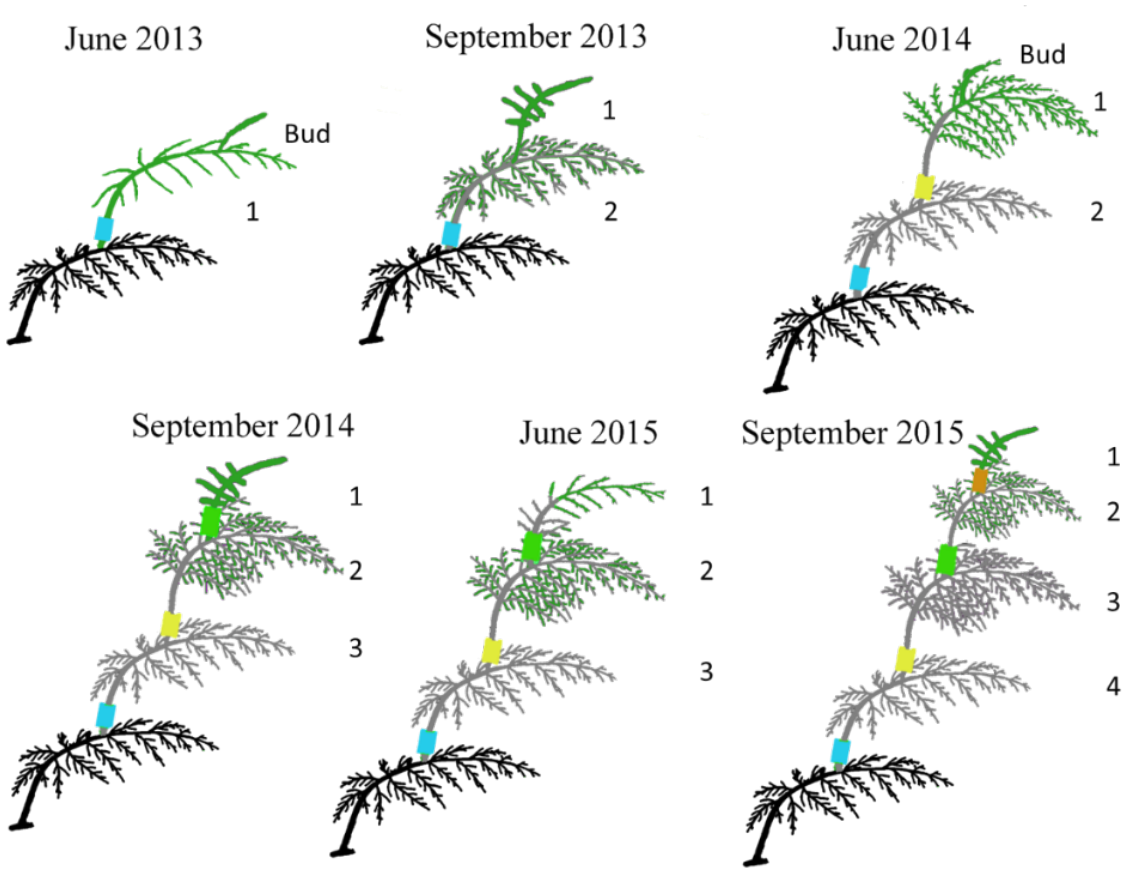

Figure 3.2: Schematic diagram illustrating the growth form and development over time of a shoot of *Hylocomium splendens*. The green segments represent new growth in each time period (modified from Økland (1995)). Segment number from the tips of the moss are indicated on the right. Segments were marked with a new colour as they emerged.

3.2.6 Statistical analyses

All the mixed-model analyses from this section were conducted using the library *lme4* (Bates et al. 2014) and *lmerTest* (Kuznetsova et al. 2015) in R (R Development Core Team 2016). Random effects in the models accounted for the hierarchical nesting of my sampling design (moss shoots when applicable, nested within SU, nested within plots, nested within forests, nested within blocks). Tukey Honest Significant Difference (HSD) post-hoc tests were used when applicable. Specific details regarding the coding structure of each model are provided as footnotes to the statistical tables presented in the results section. Leaf litter cover (percent) on the SUs was compared among forest types and treatments using a generalized linear model with a binomial family and a logit link function (Bates et al. 2014).

3.2.6.1 Moss growth, greenness, and reproductive responses to forest type and leaf litter

Moss biomass was transformed using a natural logarithm for these analyses to comply with the assumptions of normality and homoscedasticity. This transformation was selected because of the exponential growth of moss shoots over time. First, I used a linear mixed effect model to confirm that there were no initial differences in the biomass of the 2012 segment according to treatment ($p = 0.6703$), forest type ($p = 0.6193$), or their interaction ($p = 0.2600$, Table A4.2). I then used linear mixed models to test for the effect of the interaction between forest type, leaf litter treatment, and sampling year on moss biomass. A significant three-way interaction was followed by conducting separate models for each time period with a Bonferroni correction (Crawley 2007). I then investigated how total moss growth between June 2013 and August 2015 (no data transformation used as the distribution of this variable complied with assumptions) varied according to leaf litter cover (%) as a continuous variable, canopy openness (%), and forest type using mixed effect models. I used Akaike's Information Criterion (AIC) (Crawley 2007) to select the most parsimonious model.

I analyzed the moss greenness in 2012 and in 2015 using linear mixed effects models. A fixed effect of 2012 colour accounted for the influence of previous moss colour (and therefore health and growth) on 2015 colour, and adding this factor improved the model significantly based on AIC. For the 2015 data, I also ran two separate models, one using leaf litter treatment as a categorical variable, and another one using leaf litter as a continuous variable. Both linear and quadratic effects of leaf litter were included in the model.

In order to assess the impacts of forest type and leaf litter on moss fitness, I counted the number of sporophytes present on the samples following the destructive harvest of August 2015, and calculated the total number of sporophytes per transplant. I included the number of moss shoots per transplants (5-13, average of 9) as a fixed effect in the statistical analyses. I wanted to focus only on new sporophyte production that would have been affected by my experimental manipulations, so I only included sporophytes found on steps that formed during the summer of 2013 onwards (above the yellow bead in Figure 3.2). I used generalized linear mixed models with a Poisson distribution for zero-inflated and overdispersed count data from the package *glmmADMB* (Skaug et al. 2014). To account for possible phenotypic differences among forest types in sporophyte production, I ran one baseline model focusing only on the procedural controls to assess differences associated with forest types, and one model focusing only on the transplant data to investigate leaf litter impacts on mosses that all originated from spruce stands. My sample size was not large enough to include the interaction term between forest types and leaf litter treatments in the transplant model. I therefore decided to use leaf litter cover as a continuous variable instead of treatment, as this variable accounted for the difference in leaf litter cover in the ambient treatments.

3.2.6.2 Moss architecture response to forest type and leaf litter

I analyzed the average number of segments per moss shoot according to forest type, leaf litter treatment, and sampling year using linear mixed-models. Next, I compared the number of side segments produced per moss shoot at the end of the experiment, according to forest types and treatment. These two variables were square root transformed prior to analysis to comply with the assumptions of normality and homoscedasticity (Crawley 2007), and because a Poisson model for count data could not be used on averaged (non-integer) data. Lastly, I investigated how production of new moss segments between June 2013 and August 2015 (no data transformation used) varied according to the continuous variables of leaf litter cover (%) and canopy openness (%), and forest type, and used AIC (Crawley 2007) to select the most parsimonious model from a series of nested models starting with the three-way interaction.

For the last year of the experiment (September 2014-August 2015), I calculated the proportion of moss biomass growth that was allocated to either the production of new segments, or to the elongation and branch expansion of existing segments. In order to do so, I only used

measurements from the dominant chain of segments for mosses measured in September 2014 and August 2015, as I was able to identify of each segment with certainty. Any negative growth values for individual segments were replaced with zeros, and moss shoots that presented a total growth that was either negative or zero were removed from the analyses. Of the 1136 moss shoots that were measured both in September 2014 and August 2015, only 55 showed no positive growth (mostly due to mechanical damage and branch loss). Out of those 55 shoots, 83% came from the leaf litter addition treatments in birch and spruce stands or from the ambient treatment in birch stands, which suggested that mosses under leaf litter were more fragile. The proportion allocated to new growth was compared among forest types and leaf litter treatments using a generalized linear mixed effect model with a binomial family and a logit link function (Bates et al. 2014).

3.2.6.3 Physical, chemical, and biotic mechanisms of leaf litter impacts on mosses

I used linear mixed-models to compare *H. splendens* and *P. schreberi* growth (final biomass data from September 2015) according to the four treatments from the leachates experiment using plot as the random effect. Initial moss measurements (September 2014 for *H. splendens* and June 2015 for *P. schreberi*) were also included in the model to incorporate pre-experiment differences in moss size. To comply with assumptions of normality and homoscedasticity, I used a square root transformation on the *H. splendens* biomass data, and a natural logarithm transformation on the *P. schreberi* data. Fungal infection was quantified through visual estimates of the fungal hyphae cover (%) on the SUs of the moss transplant experiment in August 2015. A Spearman rank correlation was used to investigate the relationship between leaf litter and fungus cover (%), as their relationship was monotonic and non-linear.

3.2.6.4 Ecosystem contributions of mosses

I estimated the total biomass produced (ANPP) by *H. splendens* at the stand scale under the different combinations of forest types and leaf litter treatments. The average growth per shoot per year (g moss shoot^{-1}, measurement for 2014 to 2015 only), moss cover (%), and moss shoot density (growing points cm^{-2}) were used to produce those estimates. I then used values of annual net primary productivity (ANPP) estimated for my study sites (Melvin et al. 2015) to compare the contribution of *H. splendens* to stand-level ANPP.

3.3 Results

3.3.1 Leaf litter cover

In both forest types, the leaf litter addition treatments had the highest leaf cover, nearing 100% every year, and the exclusion treatments had the lowest leaf cover, near 0% (Table A4.3; Figure 3.3). Leaf litter cover was higher in the ambient treatment and procedural control of birch than of spruce stands. In both cases, there was more variability in leaf litter than in the manipulated treatments (Figure 3.3).

3.3.1 Moss growth, health, and reproductive responses to forest type and leaf litter

3.3.1.1 Moss growth

We found a significant three-way interaction between sampling time, forest type and leaf litter treatment on the average biomass per moss shoot (Table A4.4a; Figure 3.4a). One year after the start of the experiment, the only difference observed was that transplanted mosses were larger in spruce stands compared to birch stands (Table A4.4b). This trend was consistent with mosses from the procedural controls being significantly larger in spruce (9.8 mg ± 0.5 SE) than in birch (6.9 mg ± 0.4 SE) stands. Two years after the start of the experiment, transplanted mosses were still larger in spruce than in birch stands and moss shoots in the addition and ambient leaf litter treatments had a lower mass than the ones in the exclusion treatments and the procedural controls (Table A4.4c). At the end of the experiment, there was an interaction between forest type and treatment (Table A4.4d). Mosses were about 1.7 times larger in treatments with low leaf litter inputs (procedural control, exclusion and ambient in spruce, and procedural control and exclusion in birch) than in the treatments with a high leaf litter cover (ambient and addition in birch, and addition in spruce). The detrimental impact of leaf litter on moss growth is especially prominent in the treatments with added leaf litter; mosses in these treatments experienced a very low growth rate between September 2014 and 2015 (Figure 3.4a).

I found a negative impact of leaf litter cover as a continuous variable, an effect of forest type (larger mosses in spruce stands), and an interaction between canopy cover and forest type (Table A4.5; Figure 3.5) on moss growth between 2013 and 2015. There was a large spread in the range of moss growth at low leaf litter covers, with a tendency for mosses to be larger in spruce than in birch stands (Figure 3.5a). Moss growth was higher in birch stands with lower canopy cover and therefore greater light availability, while the opposite relationship was apparent in spruce stands (Figure 3.5b).

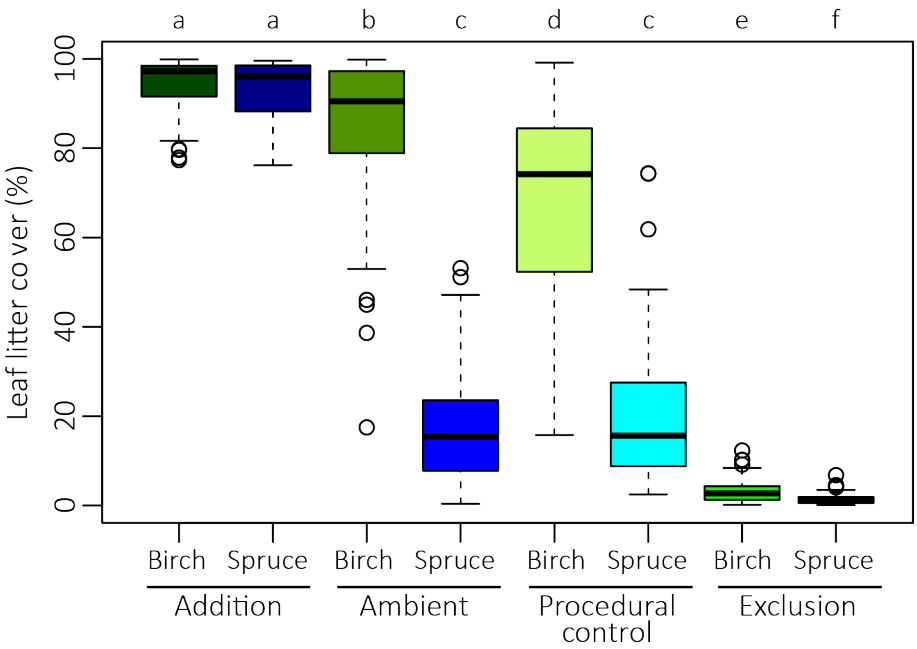

Figure 3.3: Leaf litter cover on the sampling units from 2012 to 2015. The colours represent forest type and leaf litter treatment. Medians are indicated by the black horizontal lines, boxes include 25–75% quantiles of the data, while whiskers include 5–95%. Outliers are shown as open circles. Different letters indicate that the treatments were significantly different (Tukey HSD post-hoc test, $\alpha < 0.05$).

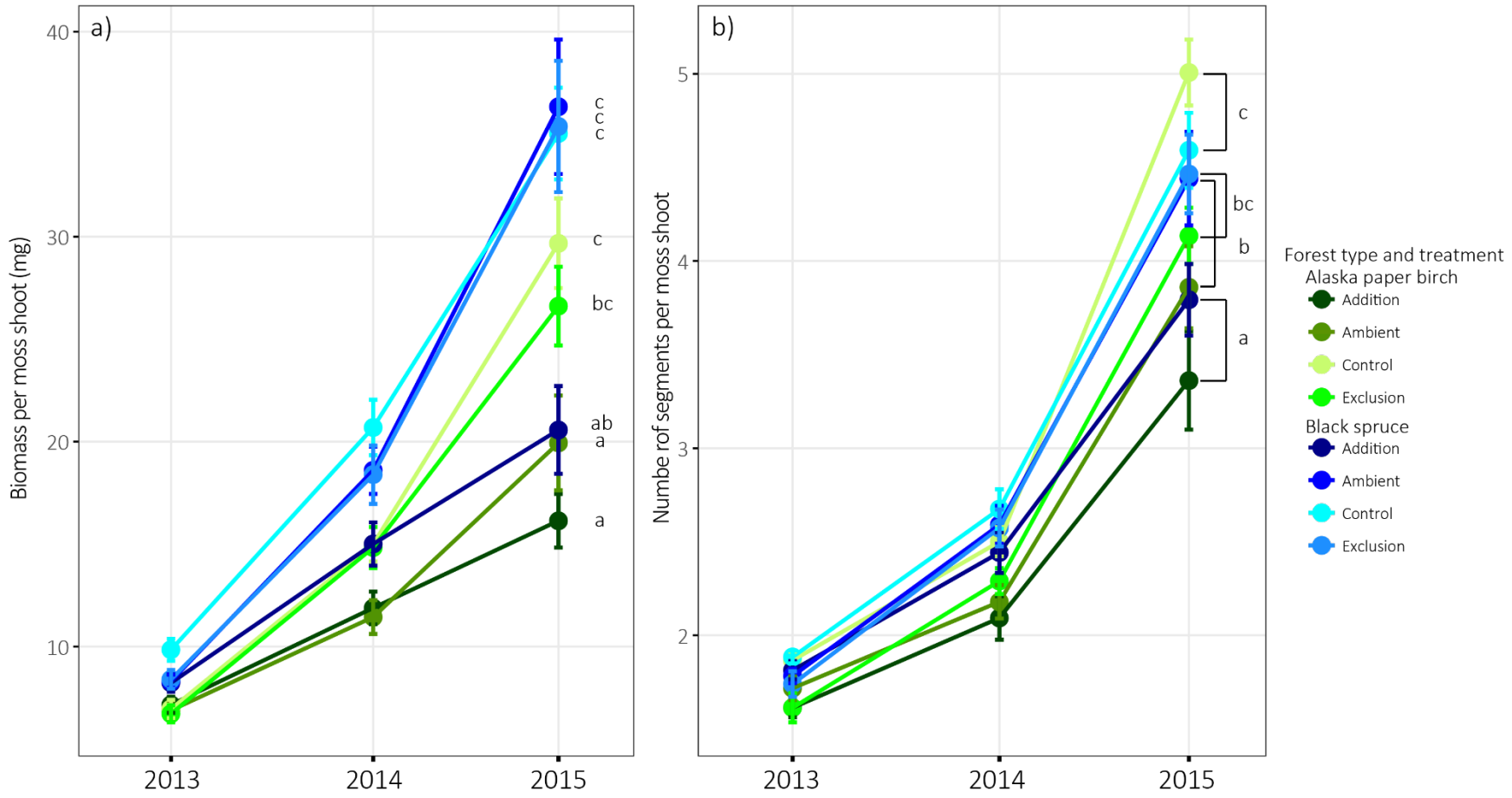

Figure 3.4: a) Average biomass of individual moss shoots (± standard error) and b) number of segments per moss shoot (± SE) according to sampling time (September 2013, September 2014, and August 2015). Colours indicate forest type and leaf litter treatment. Different letters indicate that the treatments were significantly different in 2015 (Tukey HSD post-hoc test). Note that data were transformed for statistical analysis using the panel (a) natural log and panel (b) square root.

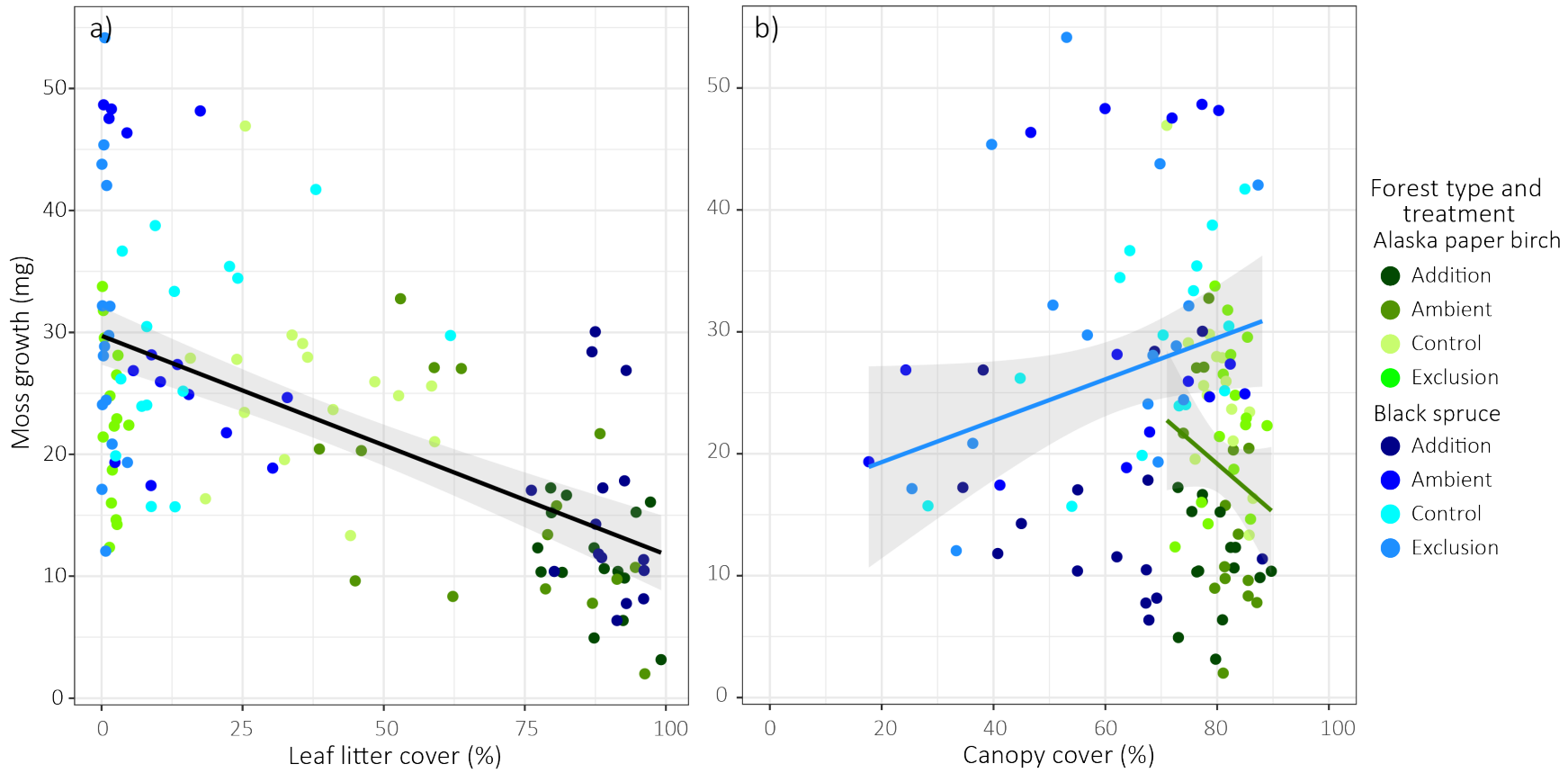

Figure 3.5: Moss growth between June 2013 and August 2015 (mg) in relation to a) leaf litter cover (%) and b) canopy cover (%). Points are averages of moss shoot growth per transplant, and the colour represents forest type and leaf litter treatment. The lines represent the significant linear relationship between moss growth and (a) leaf litter cover, and (b) the interaction among forest type (spruce in blue, birch in green) and canopy cover with a 95% confidence interval shading (see Table A4.5 for statistics).

3.3.1.2 Moss greenness

In September 2012, i.e. after mosses were transplanted and before the leaf litter treatments were applied, mosses in the procedural controls were greener than in the transplants (Table A4.6a) indicating a possible stress from the transplantation. At the end of the experiment (August 2015), mosses in ambient and experimental leaf litter addition treatments were less green than mosses in procedural controls or in leaf litter exclusion treatments (Table A4.6b), regardless of forest type (Table A4.6b). Relationships of moss greenness with leaf litter and canopy cover expressed as continuous variables showed significant quadratic effects of leaf litter cover (Table A4.7). As with moss growth, there was a lot of variability in moss greenness near 0% leaf litter cover. Moss greenness increased with moderate amounts of leaf litter (up to about 25-30% cover) and then decreased, reaching the lowest values near 100% leaf litter cover (Figure 3.6). There was a trend for mosses to be greener in birch than in spruce stands, but the effect of forest type was not significant (Table A4.7).

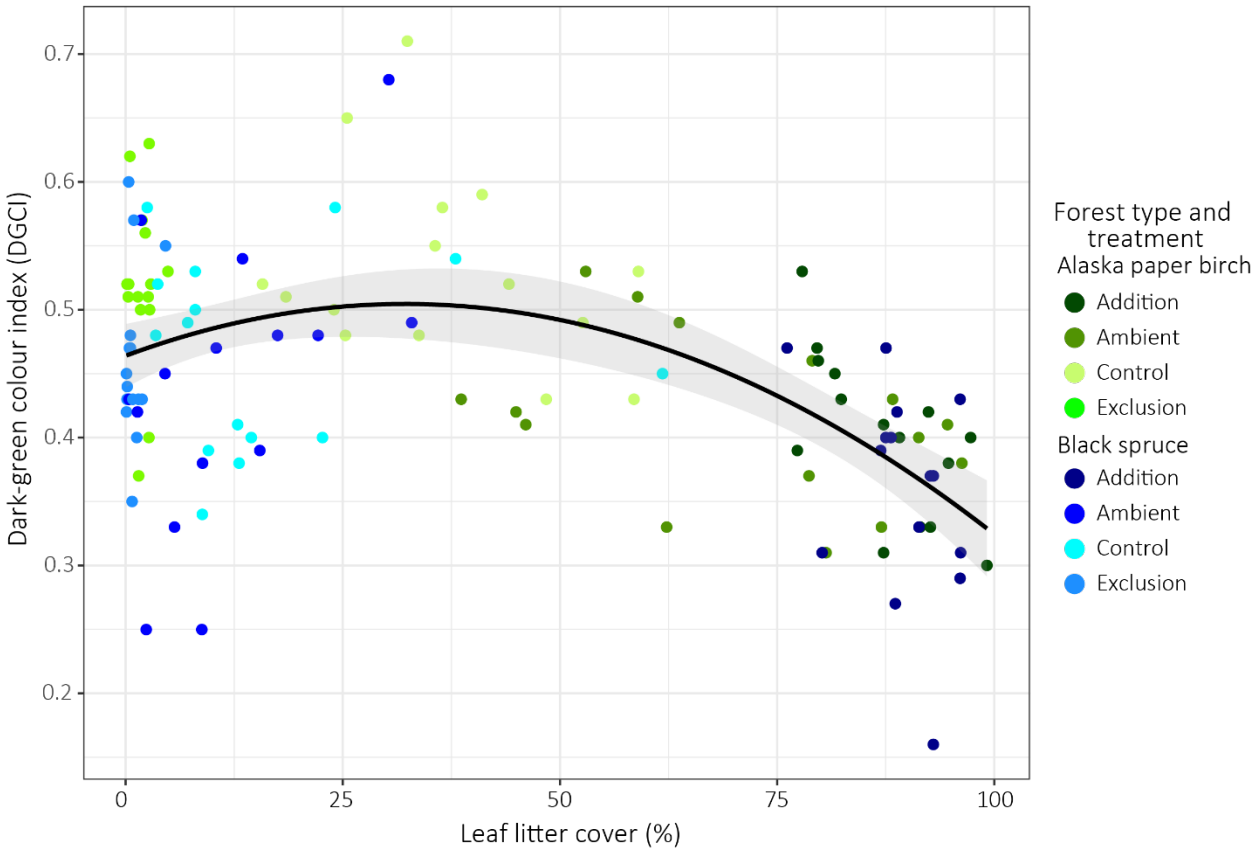

Figure 3.6: Moss greenness (modified DGCI) in relation to leaf litter cover (%). Points present the average of moss shoot greenness per transplant, and different colours represent the different treatments and stand types. The trend line is the quadratic regression line with shading to represent the 95% confidence interval.

3.3.1.3 Sporophytes

Hylocomium splendens segments in the procedural controls produced at least one spoprophyte on about 1.8% and 6.1% of measured moss segments in birch and spruce stands, respectively. In the transplanted SUs not subjected to leaf litter inputs, these values ranged between 3.6 and 6.1%, while in transplants with experimental and ambient litter inputs, these values ranged between 0.2 and 0.6%.

Sporophyte production per transplant between 2013 and 2015 was slightly lower in the procedural controls in birch than in spruce stands (Table A4.8a; Figure 3.7). Leaf litter cover on the transplants had a strong negative impact on sporophyte production (Table A4.8b). Sporophyte production was higher in transplants growing in spruce compared to birch stands (Table A4.8b), but this effect may be driven by the larger number of transplants affected by leaf litter in birch than in spruce stands. Indeed, sporophyte production in the exclusion treatment was similar in both forest types. Regardless of the forest type, all transplants with ambient and experimental leaf litter addition were either unable to produce or produced few sporophytes (Figure 3.7). In both procedural controls and transplants, there was no significant relationship between the number of sporophytes and the number of individual moss shoots included in the counts (Table A4.8a-b).

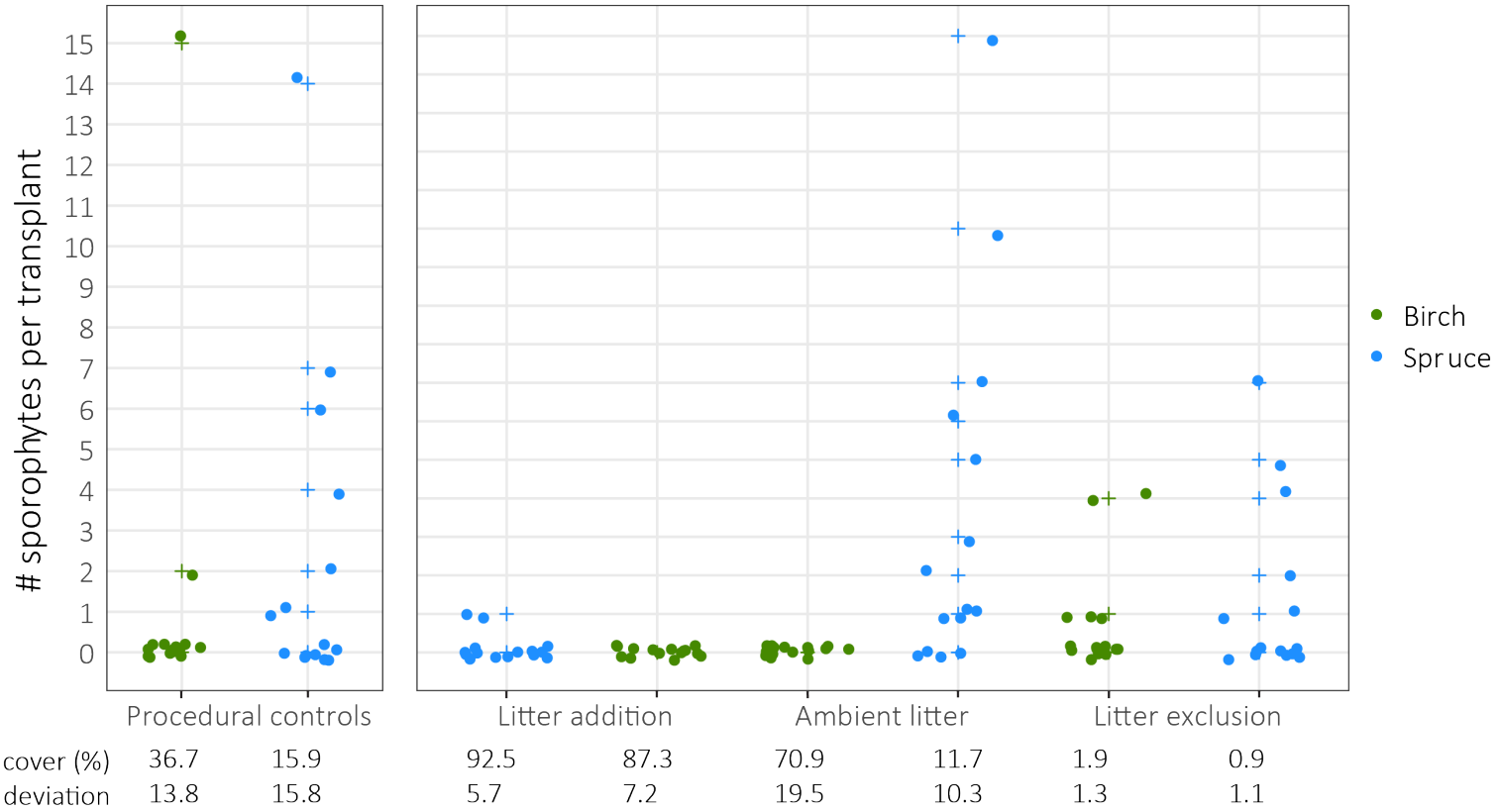

Figure 3.7: Number of sporophytes on moss segments produced between 2013 and 2015 per transplant according to treatment and forest type (birch: green, spruce: blue) (n = 15 per forest type × treatment) in August 2015. Procedural controls are in the left panel, and the transplants are in the right panel. Treatments of moss transplants have been ordered according to their leaf litter cover. For each treatment and forest type, the average leaf litter cover (%) of August 2015 is listed along with standard deviation. Plotted points have been randomly offset to avoid overlapping points around the central crosses that represent the actual number of sporophytes.

3.3.2 Moss architecture response to forest type and leaf litter

3.3.2.1 Moss segment production and multiple growing points

The number of segments per moss shoot was not affected by the three-way interaction between forest type, leaf litter treatment, and sampling time ($p = 0.1350$; Table A4.9; Figure 3.5b). There was however a significant interaction between treatment and sampling time (Table A4.9). At the start of the experiment, the number of segments per moss shoot was similar among all treatments (1.8 ± 0.02 SE segments). At the end of the experiment, mosses in the leaf litter addition treatments had the fewest segments (3.6 ± 0.16 SE), followed by the ambient (4.1 ± 0.2 SE) and exclusion treatments (4.3 ± 0.1 SE), and mosses in the procedural controls (4.8 ± 0.1 SE) had the most segments (Figure 3.5b). Mosses in the procedural controls of birch stands tended to produce more segments than in spruce stands, while mosses in the procedural controls of spruce stands tended to be larger (differences not significant). This suggested slightly different moss architectures between forest types, with mosses in birch stands producing more segments, but that individual segments were smaller than in spruce stands (Figure 3.4). When looking at the effects of leaf litter and canopy cover as continuous variables, only leaf litter cover negatively affected the number of segments per moss (Table A4.5b). Frequent variation in the annual branching cycle of *H. splendens* was found in interior Alaska; 22.0% of samples in 2013-2014 and 24.7% in 2014-2015 produced a least one segment with multiple segment growth points. Production of multiple segments in one year along the main axis of the moss seemed to be negatively affected by leaf litter inputs, both experimental and natural (Table A4.10). The occurrence of multiple growing points was high and similar for procedural controls and leaf litter exclusion treatments in 2014-2015 (26.6-36.8%).

3.3.2.2 Moss biomass allocation to new and existing segments

Litter treatments had a significant impact on how *H. splendens* mosses allocated biomass to either the production of new segments or to the elongation of old segments, but this was not affected by forest type or the interaction between forest type and treatment (Table A4.11). The typical allocation of biomass, as estimated from the procedural controls, was 20.0% ± 1.8 (SE) to new segments, and 79.2% ± 2.1 (SE) to elongate and widen existing segments. Mosses in the addition treatment decreased their allocation of biomass to new segments to 11.9% ± 1.7 (SE). Allocation of biomass to new segments was similar to the procedural controls in the exclusion 20.7% ± 1.8 (SE) and ambient treatments 16.8% ± 2.0 (SE). In the field, I also noticed that

mosses under a high leaf litter cover tended to produce long and narrow new segments with very few side branches, likely as a response to the shading effect of leaf litter cover (Figure A4.1).

3.3.3 Physical, chemical, and biotic mechanisms of leaf litter impacts on mosses

The treatments used to test the effects of leaf leachates vs. leaf structures on moss growth had no significant effect on the biomass of *H. splendens* (Table A4.12a), but significantly affected the biomass of *P. schreberi* (Table A4.12b). Mosses in the control and leachate treatments tended to be larger than mosses in the leached leaf litter and leaf litter addition treatments for *P. schreberi*, and a similar non-significant pattern was observed for *H. splendens* (Figure 3.8). Given the short duration of this experiment, moss biomass was also positively correlated with initial shoot size for both *H. splendens* and *P. schreberi* (Table A4.12).

Starting in 2014, I noticed extensive fungal hyphae colonization of the moss surface below both natural and experimental leaf litter inputs in the main moss transplant experiment, and this presence became prevalent in 2015. There was a very strong correlation between leaf litter cover and fungal hyphae cover on the SUs in August 2015 (Spearman's $\rho = 0.87$, $p < 0.0001$; Figure 3.9). This fungal cover on the mosses may represent a biotic mechanism for the negative impacts of leaf litter growth that may require a few years (two years in this case) to develop. Indeed, there was a significant linear relationship between moss growth over the course of the experiment and fungus cover on the SUs ($F_{1, 92.7} = 75.329$, $p < 0.0001$), suggesting that increased fungal presence may be another mechanism through which leaf litter affects moss growth.

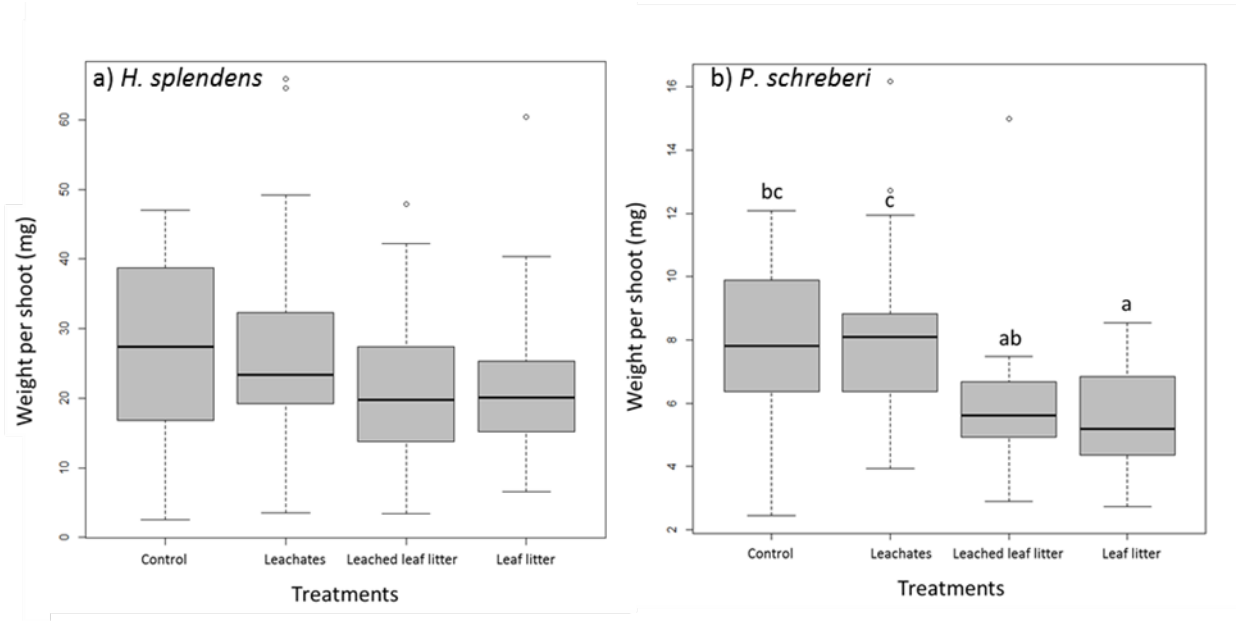

Figure 3.8: Final average weight per shoot (mg, September 2015) of *Hylocomium splendens* (a) and *Pleurozium schreberi* (b) according to treatment. Medians are indicated by the black horizontal lines, boxes include 25–75% quantiles of the data, while whiskers include 5–95%. Outliers are shown as open circles. Letters above the whiskers indicate that the groups were significantly different using a Tukey HSD post-hoc test ($\alpha < 0.05$).

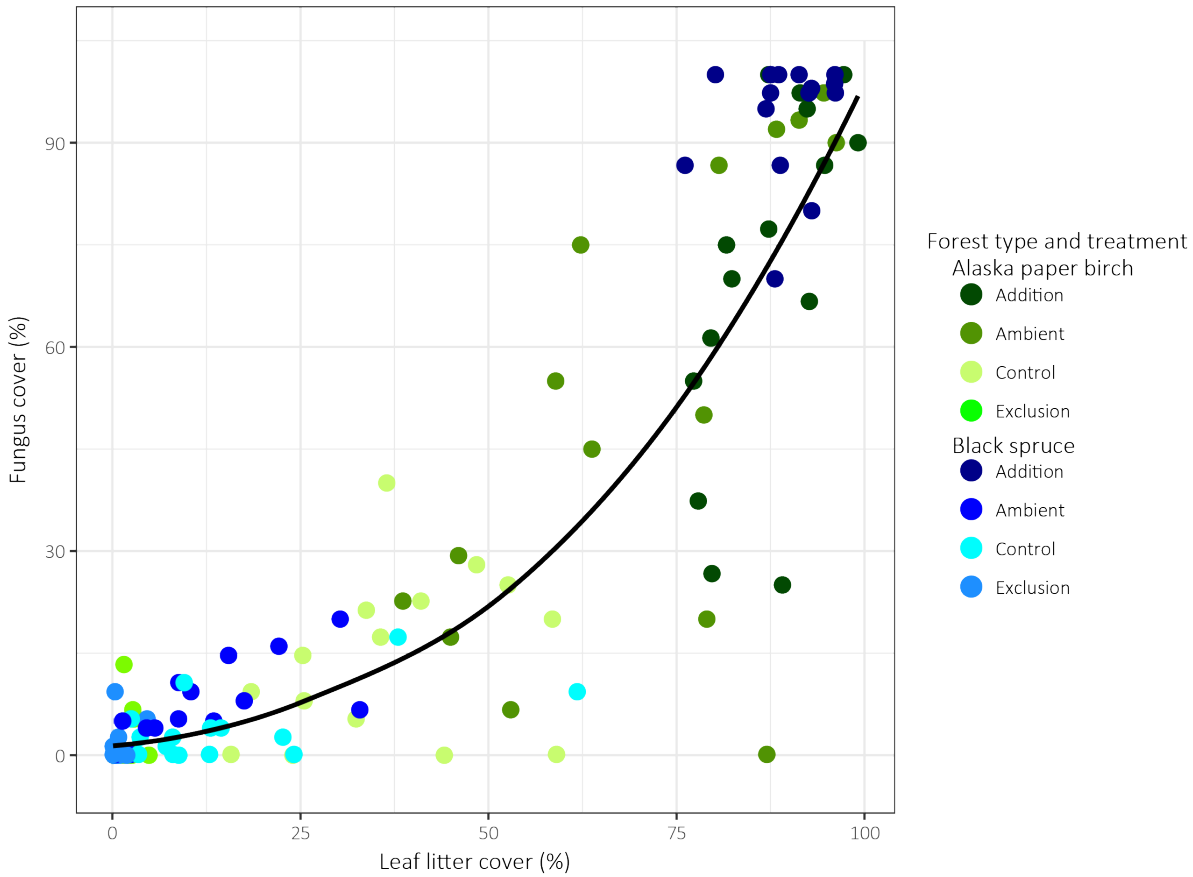

Figure 3.9: Fungus cover (%) according to the 2015 leaf litter cover (%) on the sampling units. Each point represents a sampling unit and the colours represent forest type and leaf litter treatment. The curve was fitted with LOESS (locally weighted scatterplot smoothing) with a smoothing parameter of 1.

3.3.4 Ecosystem contributions of moss growth

The first moss segments marked in June 2013 (blue bead, Figure 3.2) grew by about 3.4 mg ± 0.14 SE (39% weight increase) between September 2014 and August 2015 (paired t-test, $t_{1135} = -24.425$, $p < 0.0001$), despite these segment being in their 4th year. I therefore included these segments in my estimates of total biomass produced.

There was a large difference in the contribution of *H. splendens* to ANPP between birch and spruce stands (Figure 3.10). I found that moss ANPP in procedural controls was 0.851 g m^{-2} year^{-1} in birch stands, and 54.2 g m^{-2} year^{-1} in spruce stands (Table 3.1). The extensive moss cover and higher density of moss growing points per area in spruce stands compared to birch stands were the major drivers of the differences in moss ANPP. I estimated the contribution of *H. splendens* to stand level ANPP to be about 0.18% in birch stands, and about 22% in spruce stands (Table 3.1). Mosses transplanted in the birch stands produced amounts of biomass that were more similar to the procedural controls in birch than to the spruce stands where they originated (Table 3.1; Figure 3.10). In both forest types, experimental and ambient leaf litter inputs lead to large decreases in moss biomass production and my extrapolated contribution to stand level ANPP, with estimates being between 38 and 62% of those of the procedural controls (Table 3.1; Figure 3.10).

Table 3.1: Estimates of moss biomass production per year (g m^{-2} $year^{-1}$) estimated from individual moss shoot growth in 2014-2015 and the number of growing points, moss cover, and moss shoot density (number of growing points).

Treatment	Block	Moss cover (%)	Moss density (growing points m^{-2})	Biomass production per sample (mg $shoot^{-1}$ $year^{-1}$)	Growing points per sample	Biomass production (g m^{-2} $year^{-1}$)		Percent of annual ANPP[1]
Alaska paper birch, ANPP estimates (g/m^2/year)[a]								**465**
Procedural control	A	1.8	138	15.6	2.15	1.00		
	B	4.1	244	15.8	2.69	1.43	0.82	0.18
	C	0.1	6.42	12.6	2.49	0.03		
Litter addition	A	[b]	[b]	3.51	1.88	0.26		
	B			4.26	1.57	0.66	0.31	0.07
	C			5.16	1.85	0.02		
Ambient litter	A	[b]	[b]	6.58	1.67	0.55		
	B			8.47	2.16	0.96	0.51	0.11
	C			10.5	2.35	0.03		
Litter exclusion	A	[b]	[b]	9.33	1.76	0.73		
	B			10.8	1.79	1.48	0.75	0.16
	C			15.8	2.37	0.04		
Black spruce, ANPP estimates (g/m^2/year)[a]								**247**
Procedural control	A	87.6	12100	14.7	1.75	101		
	B	51.2	4570	12.8	1.57	36.5	54.2	22.0
	C	45.6	3800	17.8	2.76	24.5		
Litter addition	A	[b]	[b]	6.33	1.88	40.8		
	B			2.41	1.85	5.94	21.2	8.60
	C			7.93	1.78	16.9		
Ambient litter	A	[b]	[b]	13.4	1.71	94.7		
	B			13.4	1.85	33.1	56.1	22.7
	C			25.9	2.44	40.4		
Litter exclusion	A	[b]	[b]	11.0	1.49	89.3		
	B			15.6	2.20	32.5	54.1	22.0
	C			24.6	2.30	40.6		

[a] Annual net primary productivity (ANPP) estimates from Melvin et al. (2015) for the tree data. I added my estimates of average moss NPP from the procedural controls to get the complete ANPP for each stand type.

[b] The values are the same as those presented for the first treatment.

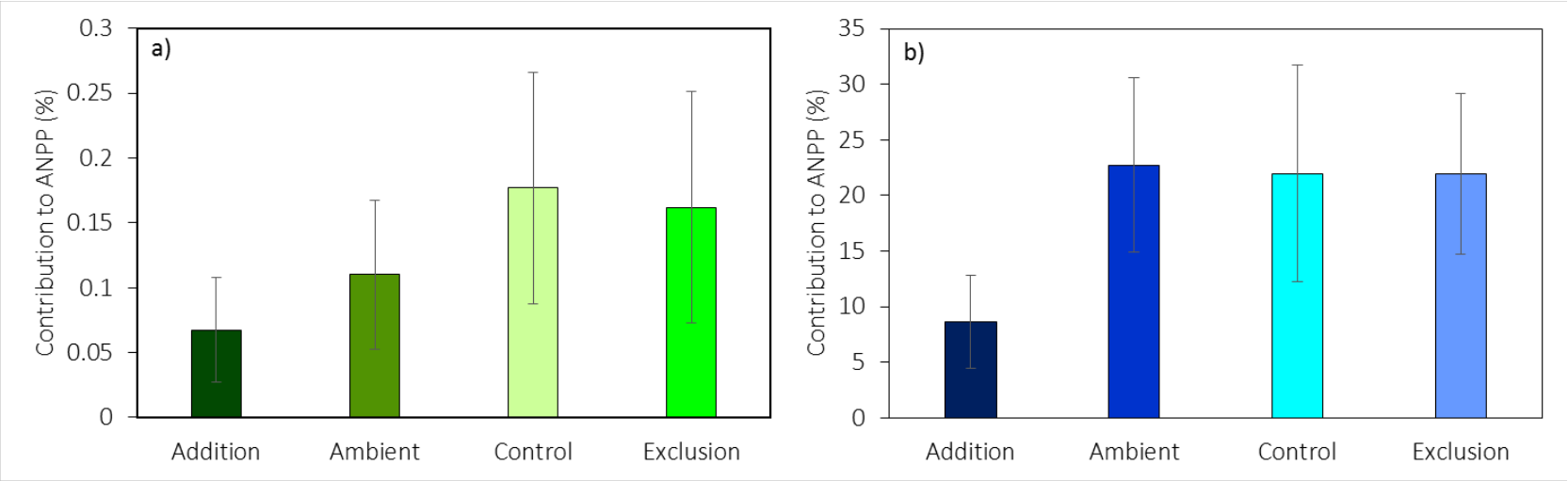

Figure 3.10: Estimated contribution of *Hylocomium splendens* to total stand annual net primary production (ANPP, average % ± SE) according to litter treatment in a) Alaska paper birch stands and b) black spruce stands. Note the different *y*-axis scales used in the two panels; see Table 3.1 for calculation details.

3.4 Discussion

Furthering our understanding of the mechanisms driving moss abundance in boreal forests is important because of their key role in plant-soil feedbacks associated with thick organic layers (Johnstone et al. 2010a, Turetsky et al. 2012), as well as their contribution to ecosystems processes such as ecosystem ANPP (Bond-Lamberty et al. 2004, Wardle et al. 2012), photosynthesis (Goulden and Crill 1997), and N_2-fixation (DeLuca et al. 2002, Chapter 3). Leaf litter inputs associated with the dominant tree species in coniferous and broadleaf deciduous forests were found to be key drivers of moss growth and may explain the discrepancy in moss abundance among those two stand types. Experimental results from this study show that the effects of leaf litter on the growth and health of the feather moss *H. splendens* are much greater than the effects of differences in environmental conditions among stand types, thus demonstrating that leaf litter is the critical mechanism excluding mosses from deciduous stands, despite potentially beneficial nutrient inputs from leaf litter. These results support the long-standing hypothesis of the detrimental effect of deciduous broadleaf litter on mosses (e.g. Van Cleve et al. 1983a), and further elucidate the role of plant-plant interactions in maintaining two main stability domains of the boreal forest, deciduous and coniferous stands (Johnstone et al. 2010a). In black spruce stands, low leaf litter inputs favour high moss productivity that produces thick organic layers, while in deciduous stands, leaf litter inputs maintain low moss cover and productivity, thus keeping shallow organic layers. Indeed, ANPP of *H. splendens* alone accounted for up to 22% of the total annual stand level productivity in spruce stands, but accounted for a negligible proportion in birch stands (< 1%). Addition of Alaska paper birch leaves on *H. splendens* transplants lead to significant reductions in moss growth, biomass accumulation, sporophyte production, and changes in moss colour and architecture in both birch and black spruce stands.

Two years were required before the impact of leaf litter treatments on mosses became apparent, and litter effects did not override stand differences as the major driver of moss growth until after three years of experimental treatment. Time lags in moss responses to litter treatments may reflect the time required to accumulate about three layers of leaves, an amount similar to that found on the forest floor of mature deciduous stands (Landhäusser and Lieffers 2002, Lieffers et al. 2002), as my input rate added approximately one full layer of leaves every year. At

the end of the experiment, mosses subjected to low leaf litter treatments (procedural control, exclusion and ambient in spruce, and procedural control and exclusion in birch) were on average 1.7 times larger and had produced approximately one more segment than mosses in the high leaf litter cover treatments (ambient and addition in birch, and addition in spruce).

Leaf litter cover (%) had a strong linear and negative impact on total moss growth and segment production over the course of the moss transplant experiment. Despite the high variability in moss growth at low leaf litter cover, results of this experiment do not support the hypothesis that intermediate inputs of birch leaf litter may have a stimulating effect on *H. splendens* growth due to leaching of sugars or nutrients from fallen leaves (Oechel and Van Cleve 1986, Sveinbjörnsson and Oechel 1992). Annual inputs of birch leaf litter in this study (224.8 g dry leaf litter m^{-2} $year^{-1}$) were high compared to the input rate of 75 g m^{-2} $year^{-1}$ in the subarctic forest of Scandinavia, where positive impacts of leaf litter on moss-associated N_2-fixation have been reported (Sorensen and Michelsen 2011). A potential uptake in carbohydrates and nutrients, along with the need for more chlorophyll to compensate the lower light availability, may help explain the result from the current study that moss greenness seemed to increase at moderate leaf litter covers up to 25 to 30%. At high leaf litter covers, moss greenness decreased, which was consistent with field observations of brown mosses. Declines in moss greenness, along with low moss growth in the leaf litter addition treatments indicated that the longer term effects of high leaf litter are likely to lead to moss mortality. Leaf litter not only affected moss growth directly, but also affected resource allocation to old vs. new segments, and reproductive efforts. Investments in sexual reproduction through sporophyte production were almost completely eliminated and there was a decrease in the frequency of production of multiple segments in a year under ambient and experimental leaf litter inputs. Allocation of biomass between elongation of existing segments and production of new ones was also altered by leaf litter treatments, with a decrease in allocation to the new segments from 20% to 12%. Indeed, mosses under leaf litter either produced fewer segments or produced linear segments with no side branches, a morphological response that was also observed by Startsev et al. (2008). Therefore, it seems likely that deciduous broadleaf litter is the key mechanisms driving differences in moss growth and abundance among deciduous and coniferous stands.

Leaf litter impacts have mostly been associated with physical and mechanical impacts such as shading, barrier to vertical growth, and crushing, as well as biotic interactions, rather

than chemical impacts (Van Cleve et al. 1983b, Beatty and Sholes 1988, Startsev et al. 2008). The addition of birch leachates alone had no effect on moss growth, while application of leached leaves had an impact on growth of *Pleurozium schreberi* similar to that of natural leaf litter addition. In contrast, in a growth chamber experiment, Startsev et al. (2008) found that aspen leachates and shading had additive negative impacts on feather moss growth and survival comparable to the application of aspen leaf litter on the moss. Aspen leaves are known to contain phenolic compounds (Taylor et al. 1996, Erwin et al. 2001), which can have allelopathic effects on moss (Légaré et al. 2005, Startsev et al. 2008), but allelopathic chemicals have not been tested for Alaska paper birch. There is also a possibility that my leachates application was either too infrequent or diluted to produce an effect in only one year. It is interesting however to note that *P. schreberi* had a clearer negative response to the short term leaf litter inputs than *H. splendens*, suggesting that the two species of feather moss may react differently to changing environmental conditions. As both species are often co-dominant in boreal forests, additional work to understand the response of *P. schreberi* to leaf litter and canopy impacts is necessary. Evidence from my experiment and the literature suggest that, even if feather mosses are considered shade tolerant species (Oechel and Sveinbjörnsson 1978, Sulyma and Coxson 2001), the addition of a continuous layer of deciduous leaf litter may completely inhibit their photosynthesis and growth (Startsev et al. 2008). Irrespective of forest type, high covers of leaf litter promoted fungal covering of mosses, which may have amplified the detrimental effects of leaf litter on mosses. Aspen leaf litter can release sugars that may be assimilated by mosses (Gupta 1977, Startsev et al. 2008), and it is likely that birch leaf litter has a similar impact. Alaska paper birch leaf litter has high N and moderate phosphorus (P) content (Melvin et al. 2015). Parasitic fungi and mosses are known to coexist (Davey and Currah 2006, Davey et al. 2016), and it is possible that an increase in nutrient availability brought about by leaf litter additions or a change in abiotic conditions such as moisture and light availability, may have unbalanced the competition between fungi and mosses, thus promoting fungal hyphae growth to the detriment of *H. splendens*. Leaf litter inputs and increases in nutrient availability may have altered trophic interactions in the bryosphere (Kardol et al. 2016), for example by promoting population increases of litter- and moss-grazing micro- and mesofauna (Lindo and Gonzalez 2010).

Overall, mosses in the procedural controls grew slightly better in spruce than in birch stands. The impact of canopy type was the first one that I was able to detect, only a year after the

start of the experiment. Moreover, in the absence of leaf litter on the transplants, there was a large variability in moss growth, colour and architecture, which suggests that environmental gradients can constrain, to some extent, the plant-soil feedbacks previously described. For example, at my study sites spruce stands had higher soil moisture, lower pH, and higher light availability compared to birch stands, all of which are environmental conditions likely to favour moss growth (Busby et al. 1978, Sveinbjornsson et al. 1922). Spruce stands also provide more hydraulic conductivity to help the development of the moss layer, which may have helped moss transplants acclimate faster. Canopy cover had a positive effect on moss growth in spruce stands, but a negative one in birch stands. Higher canopy cover in spruce stands may protect mosses from desiccation and maintain higher moss moisture. On the contrary, more shaded conditions in birch stands are unlikely to lead to significant improvements in moisture availability, and lower light availability would decrease the potential for photosynthesis and increase local leaf litter fall. I also observed some interesting stand level differences in moss phenotypes. First, I observed that mosses tended to produce more sporophytes in spruce than in birch stands. This could be due to better environmental conditions and higher light availability (Rydgren et al. 1998, Rydgren and Økland 2002a, Rydgren and Økland 2002b), a segment size threshold (Rydgren et al. 1998), high moss density increasing the chance for fertilization (Rydgren and Økland 2001), or genetic differences among populations (Cronberg 2002). Second, I found that mosses were greener in birch stands, at least in the procedural controls. A few different mechanisms may be at play, including higher N availability, or higher chlorophyll production due to lower light availability in my birch stands, paired with a higher production of carotenoids in spruce stands as a light protection mechanism (Marschall and Proctor 2004, Tobias and Niinemets 2010).

Feather mosses are important components of the carbon (C) cycle in boreal ecosystems, as they may produce large amounts of biomass and recalcitrant litter that builds soil organic layers and contribute up to 80% of C stored in boreal soils (Flanagan and Van Cleve 1983, Apps et al. 1993, Kolari et al. 2006). I estimated the average productivity of *H. splendens* to be low in birch stands (~0.8 g m^{-2} $year^{-1}$), while it was a significant contributor to stand productivity in black spruce stands (~54.2 g m^{-2} $year^{-1}$), which is in line with other estimates from boreal Alaska and Canada (Lindo et al. 2013). This difference was mostly due to the much higher cover of *H. splendens* found in spruce than in birch stands. Productivity in the transplants that had a similar leaf litter cover to the procedural controls was similar, indicating that mosses recovered well

from the stress of the transplantation. In both forest types, experimental and ambient leaf litter inputs on the mosses decreased moss annual productivity by more than 50%. Some interesting demographic characteristics were found in the studied *H. splendens* population. Unlike what was found in Norwegian forests (Økland 1995), aberrances in the annual branching cycle of *H. splendens* (i.e. the production of more than one growing point in one year) were relatively common in interior Alaska. Production of sporophytes on mature segments was higher (6.1% in spruce stands) than previously reported in Scandinavia (0.05% in Økland (1995) and 2.8% in Callaghan et al. (1978)). Furthering our understanding of the mechanisms controlling the production of segments on *H. splendens*, such as length of the growing season, light, moisture, or biotic interactions (competition), is critical in order to accurately estimate biomass production in geographically distinct sites or sites with very different environmental conditions.

3.5 Conclusion

In this study, the interaction between moss growth, biomass production and leaf litter controled the formation of the soil organic layer and provides experimental evidence for the role of plant-plant and plant-soil feedbacks in stabilizing successional dominance of black spruce and paper birch stands in interior Alaska (Johnstone et al. 2010a). Production of large amounts of broadleaf litter in deciduous stands is a stabilizing feedback in this type of stand, as it has larger detrimental impacts on feather moss growth than the other environmental conditions associated with this stand type, and low moss productivity promotes a more complete combustion of shallow organic layers. On the contrary, moss productivity was higher in black spruce stands and constituted an important proportion of total stand ANPP. Significant changes in the growth, health and architecture of mature shoots of *H. splendens* occurred within only three years of leaf litter manipulation. These effects appear to be largely attributable to the shading and weight of leaf litter, along with indirect effects of biotic interactions with fungi. Future increases in fire severity that lead to an increase in deciduousness in the landscape (Mann et al. 2012) will dramatically decrease moss growth through increased deciduous broadleaf litter production. Once deciduous broadleaf stands establish, leaf litter inputs are likely to prevent the recovery of stands previously dominated by feather mosses under a spruce canopy.

3.6 References

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CHAPTER 4: SPATIAL AND TEMPORAL VARIATION IN MOSS-ASSOCIATED DINITROGEN FIXATION IN CONIFEROUS AND DECIDUOUS DOMINATED ALASKAN BOREAL FORESTS

4.1 Introduction

The boreal forests of the world are an important carbon (C) sink (Deluca and Boisvenue 2012), making the productivity of these ecosystems of global importance in regulating atmospheric carbon dioxide (CO_2) concentrations (Bonan et al. 1992). Productivity in those ecosystems is often directly limited by nitrogen (N) availability (Tamm 1991, Vitousek and Howarth 1991, Cleveland et al. 1999). Biological N_2-fixation (BNF) by various bacteria, such as free-living and symbiotic cyanobacteria, is a major source of N in many ecosystems. The lack of widespread N_2-fixing symbioses with vascular plants in mature boreal forests is one of the reasons put forward to explain this N limitation (Tamm 1991, Cleveland et al. 1999). Associations between mosses and N_2-fixing cyanobacteria have been identified as a major source of ecosystem N inputs in mature Scandinavian boreal ecosystems (DeLuca et al. 2002, DeLuca et al. 2007,). There, they contribute up to 2-4 kg N ha^{-1} $year^{-1}$ (Lagerström et al. 2007, DeLuca et al. 2008, Gundale et al. 2011), a source of N that is comparable to atmospheric N deposition (Sponseller et al. 2016). Most of this newly fixed N is thought to remain within the moss layer (Hyodo and Wardle 2009, Gavazov et al. 2010) and may potentially become an available source of N to vascular plants through mycorrhizae (Parke and Linderman 1980, Carleton and Read 1991).

In boreal ecosystems, feather mosses such as *Pleurozium schreberi* (Brid.) Mitt. and *Hylocomium splendens* (Hedw.) Schimp. host N_2-fixing cyanobacteria on their leaves and stems (DeLuca et al. 2002, Ininbergs et al. 2011). BNF depends on favourable temperatures (Chapin et al. 1991, Gundale et al. 2012b), light (Gundale et al. 2012a), and moisture (Chapin et al. 1991, Zielke et al. 2005, Rousk et al. 2014), and tends to be low at sites with high N availability because BNF is an energetically demanding process (Chapin et al. 1991, DeLuca et al. 2008, Gundale et al. 2009). Abiotic conditions may therefore be important drivers of moss-cyanobacteria association BNF rates (Rousk and Michelsen 2017). Cyanobacteria communities associated with *P. schreberi* are thought to be the major contributor to BNF in Scandinavian boreal forests (DeLuca et al. 2002, Zackrisson et al. 2004), although it is increasingly recognized that the contribution of cyanobacteria on *H. splendens* to N fluxes and pools may also be substantial (Zackrisson et al. 2009). Feather mosses are prevalent in the understory of mature boreal forests around the world (Oechel and Van Cleve 1986) and they perform important

ecosystem processes because of their high net primary productivity, production of recalcitrant litter, moisture retention, and soil insulation (Turetsky 2003, Turetsky et al. 2012, Lindo et al. 2013).

Variations in moss-associated BNF have not yet been investigated across different forest canopy types. Most of the work on moss associated BNF in boreal forests has focused on Fennoscandia coniferous boreal forests with an extensive moss cover (e.g. DeLuca et al. 2002, Zackrisson et al. 2004, Zackrisson et al. 2009, Jackson et al. 2011, Gundale et al. 2012b). Boreal forests are composed of both coniferous and broadleaf deciduous stands, both of which are important in the Alaskan boreal forest. There, black spruce (*Picea mariana* Mill. B.S.P.) forests occupy about 40% of the forested landscape in interior Alaska (Calef et al. 2005) and pure deciduous forests occupy about 20% (Van Cleve et al. 1983a). Deciduous broadleaf species, such as Alaska paper birch (*Betula neoalaskana* Sarg.) or trembling aspen (*Populus tremuloides* Michx.), often dominate at the southern limits of the boreal forest, in younger stands, as well as on well-drained south-facing slopes with either deep active layers or no permafrost (Chapin et al. 2006). Forests in interior Alaska have a colder and drier climate than boreal Fennoscandia, are regularly affected by large stand-replacing disturbance such as fires (ACIA 2005), and have comparably low N deposition rates (Gundale et al. 2011); these factors may alter the patterns and ecosystem contributions of moss-associated BNF.

This landscape heterogeneity in interior Alaska is potentially critical for BNF, as broadleaf deciduous stands tend to have warmer and drier soils in the summer, higher N availability, and higher decomposition rates than coniferous forests, such as those dominated by black spruce (Légaré et al. 2005, Melvin et al. 2015). In addition, N fluxes and the distribution of N pools also differ among deciduous and coniferous stands, with larger aboveground N pools in the tree biomass of deciduous stands contrasted with larger below ground N pools in soils of coniferous stands (Melvin et al. 2015, Alexander and Mack 2016). Canopy composition is a major driver of moss abundance (Hart and Chen 2006). In interior Alaska, mature coniferous forests have a moss cover of over 80%, mostly dominated by feather mosses, while the moss cover in mature deciduous forests in only about 5% (Jean et al. 2017). This difference has been attributed to regular inputs of abundant and high-quality leaf litter inputs in deciduous stands, as the prostrate growth form and slow growth of mosses make them particularly susceptible to burial (Van Cleve et al. 1983a, Oechel and Van Cleve 1986). Indeed, increases in leaf litter cover

lead to significant decreases in moss abundance during post-fire succession (Jean et al. 2017), and a significant reduction in annual moss growth when broadleaf litter is applied experimentally (Chapter 3). Strong spatial (among sites and canopy types), and temporal (among years or months) variability in BNF is to be expected, but how much this variability may affect the overall contribution of moss-cyanobacteria to stand level N pools is unclear.

My objective is to estimate the stand-level N contribution of BNF associated with *P. schreberi* and *H. splendens* in mature Alaska paper birch and black spruce stands in interior Alaska. In order to make those estimates, I measured how moss-associated BNF rates were shaped by inter-annual variation, intra-annual variation during the growing season, and spatial variation. Additionally, I related environmental and climatic variables, such as temperature, moisture, light, and N availability, to BNF, to understand the mechanisms driving the observed patterns. If fixation per unit moss biomass is primarily limited by light, moisture, or nutrient availability, then rates of BNF per unit moss biomass should be higher in the spruce stands studied, as these stands have less canopy shading, stable and high moisture levels, and lower available N; if in contrast, temperature limits rates of BNF, then the highest rates per unit moss biomass should be observed in birch stands, where surface temperatures are warmer. Stand-level N_2 fixed (per unit area) should be much higher in spruce than in birch stands, as mosses are more abundant. If yearly or seasonal variation in weather (mostly temperature and precipitation) are driving fixation rates, then variation in those variables should override canopy or moss species effects. While I have some information on moss-associated BNF in black spruce-feather moss stands (Billington and Alexander 1978, Billington and Alexander 1983), this research will also give us insights into how the contribution of feather moss associated BNF to N cycling varies in space and time, and contrast those patterns in deciduous and coniferous boreal forests of interior Alaska. Moreover, I used stable isotope labelling to measure rates of BNF, which is expected to give much more robust estimates than the traditional acetylene reduction method (Hardy et al. 1973).

4.2 Methods

4.2.1 Study area and site description

The main field site for this research project is part of the Regional Site Network of the

Bonanza Creek Long-Term Ecological Research (LTER) program (www.lter.uaf.edu/). It is located in the Tanana Valley State Forest near Fairbanks in interior Alaska (64° 53' N, 148° 23' W). This site comprised stands that originated from a 1958 wildfire. In 2012, I established three blocks (A, B, and C) that each included adjacent black spruce (hereafter called spruce) and Alaska paper birch (hereafter called birch) stands. All birch stands had evidence of spruce presence prior to the last fire. Blocks A and B were representative of upland forests in the area, while block C was adjacent to a creek and presented a more riparian plant community. Near-surface permafrost was present in the spruce stands (~50 cm), but not in birch stands. Soil organic layer depth and pH were determined from five soil cores per stand by Melvin et al. (2015). Soil organic layers were deeper and pH was lower in spruce than in birch stands (Table 4.1). Estimates of potential N mineralization and nitrification rates (90 days) were determined on all soil organic layers and the top 10 cm of the mineral soil in the laboratory (Melvin et al. 2015) and I used it as a proxy for N availability. Nitrogen availability was generally higher in birch than in spruce stands, and higher in block C than in either block A or B. There were strong correlations between various measures of N and phosphorus (P) availability and pH (Table 4.1).

Table 4.1: Environmental variables from all spruce and birch stands.

	Year	Alaska paper birch stands				Black spruce stands			
		A	B	C	Average	A	B	C	Average
Latitude (WGS84)	-	64.881	64.876	64.896	-	64.883	64.877	64.895	-
Longitude (WGS84)	-	-148.392	-148.383	-148.453	-	-148.397	-148.383	-148.451	-
Elevation (m)	-	246	235	271	251	213	216	256	228
Slope (°)	-	12	13	9	11	6	11	4	7
Aspect	-	NE	NE	NE	-	NE	NE	NE	-
Organic layer depth (cm)	-	7.0	7.2	8.6	7.6	16.2	18.4	14.5	16.4
Air temperature (°C)[a]	2013	14.4	14.6	13.5	14.2	14.2	14.5	12.7	13.8
	2014	12.1	12.1	11.5	11.9	12.2	12.2	11.0	11.8
	2015	14.4	14.4	13.7	14.2	14.4	14.5	13.0	14.0
Soil temperature (°C) [a]	2013	6.7	7.2	8.8	7.6	4.6	4.9	2.6	4.1
	2014	7.1	7.5	8.3	7.6	4.3	5.0	3.1	4.1
	2015	7.2	8.0	8.5	7.9	4.6	5.6	2.5	4.2
Photosynthetic active radiation (W m^{-2}) [a]	2013	236.6	172.3	173.9	194.3	598.8	435.9	180.2	405.0
	2014	127.7	88.7	177.1	131.2	585.5	372.4	181.3	379.7
	2015	91.8	83.6	154.4	109.9	589.0	368.2	145.0	367.4
Relative air humidity (%)[a]	2013	63.9	65.0	68.2	65.7	66.4	67.2	73.4	69.0
	2014	73.3	74.9	74.6	74.3	73.1	75.6	78.1	75.6
	2015	70.7	71.6	71.8	71.4	70.6	72.5	76.1	73.1
Soil moisture (m^3 m^{-3}) [a]	2013	0.1	0.2	0.1	0.1	0.2	0.2	0.2	0.2
	2014	0.1	0.2	0.1	0.2	0.2	0.2	0.2	0.2
	2015	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.1
Mineral N mineralization rates (µg NH_4^+ g soil N^{-1} day^{-1})[b]	-	84.1	24.6	125.7	78.1	0	2.4	10.7	4.4
Organic layer N mineralization rates (µg NH_4^+ g soil N^{-1} day^{-1}) [b]	-	390.6	236.1	612.0	412.9	202.2	89.3	637.2	309.6
Mineral N nitrification rates (µg NO_3^- g soil N^{-1} day^{-1}) [b]	-	0.0	0.6	44.3	1.50	0.0	0.4	18.9	6.4
Organic layer N mineralization rates (µg NO_3^- g soil N^{-1} day^{-1}) [b]	-	38.9	0.9	74.3	38.0	0.3	1.4	0.9	0.9
Fibric Mehlich phosphorus (mg P g $soil^{-1}$) [b]	-	0.15	0.20	0.15	0.17	0.10	0.12	0.10	0.11
pH [b]	-	5.1	4.8	5.1	5.0	4.3	4.2	4.5	4.3

	Year	Alaska paper birch stands				Black spruce stands			
		A	B	C	Average	A	B	C	Average
Canopy cover (%)	-	87.0	85.4	83.1	85.2	63.3	67.6	76.8	69.2
Understory vegetation[c]									
Picea mariana	-	0.3	5.2	0.1	1.9	58.0	75.0	36.0	56.3
Betula neoalaskana	-	0.0	0.0	42.5	14.2	0.3	1.1	0.0	0.5
***Salix* spp.**	-	0.0	0.0	0.0	0.0	0.1	0.3	8.6	3.0
***Alnus* spp.**	-	0.0	4.0	45.0	16.3	0.0	8.1	1.4	3.2
Forbs	-	1.3	1.1	37.5	13.3	0.5	2.4	0.0	1.0
Ericaceae	-	0.0	0.0	0.0	0.0	1.5	3.2	6.2	3.6
Hylocomium splendens	-	1.8	4.1	0.1	2.0	87.6	51.2	45.6	61.5
Pleurozium schreberi	-	1.6	1.5	0.6	1.2	42.6	33.7	37.3	37.9
***Polytrichum* spp.**	-	0.8	0.1	0.0	0.3	1.7	0.4	0.6	0.9
***Dicranum* spp.**	-	1.2	0.5	0.0	0.6	0.0	0.0	0.0	0.0
Other true mosses	-	1.0	0.9	0.4	0.8	0.6	1.4	0.3	0.8
***Sphagnum* spp.**	-	0.0	0.0	0.0	0.0	0.0	0.0	9.4	3.1
Lichens	-	0.3	0.0	0.0	0.1	2.0	0.5	0.0	0.8
Coarse woody debris	-	4.6	2.6	6.3	4.5	2.2	1.7	0.0	1.3
Deciduous leaf litter	-	89.4	93.6	99.3	94.1	6.6	51.0	21.4	26.3
Moss density (shoots/100 cm^2)[d]									
Hylocomium splendens	-	79	59	95	78	158	95	87	113
Pleurozium schreberi	-	186	177	232	198	348	242	207	266
Weight per moss shoot (g)[e]									
Hylocomium splendens	-	0.04	0.03	0.03	0.03	0.02	0.02	0.03	0.03
Pleurozium schreberi	-	0.02	0.01	0.01	0.02	0.01	0.01	0.01	0.01

[a] Growing season averages of air and soil temperatures, photosynthetic active radiation, and soil moisture in blocks A, B, and C in 2013, 2014, and 2015 from the weather station in each stand. The growing season lasted from May 15th to September 9th each year, which is the average estimated by the Alaska Climate Research Center (2016).

[b] Average from 5 cores taken in each stand/block in 2013. Only the top 10 cm of the mineral soil was used. Measurements are from laboratory incubations described in Melvin (2015).

[c] Visually estimated percent cover of understory vegetation <1.5 m. Data from *P. schreberi* was adjusted based on point-intercept data collected at the same study sites collected by Melvin et al. (2012, unpublished data) and Jean et al. (2015, unpublished data).

[d] Shoot density within a 10x10x5 cm sample of monospecific moss. Not representative of moss abundance at the stand level.

[e] Weight per shoots (5 cm long) were obtained using the pooled data from all sampling periods.

In interior Alaska, the mean air temperature is -2.5°C, 65% of the annual 286 mm of precipitation falls as rain, and the growing season lasts about 135 days (Hinzman et al. 2005). The three years investigated presented contrasting weather conditions (Table 4.2), with 2013 being the warmest and driest year, and 2014 receiving about twice as much rain as 2013 or 2015. The summer of 2014 was the wettest on record in nearly a century (Alaska Climate Research Center 2016). The early growing season of 2015 was characterized by warm and dry weather (Alaska Climate Research Center 2016), which led to the second worst fire year on record, with over 5 million acres burned in the state (Alaska Interagency Coordination Center 2015). In 2012, I installed one weather station per stand (n = 6) recording data every 30 minutes (Onset Hobo H21-002). The station was equipped with sensors recording air temperature and relative humidity (Onset Hobo S-THB-M002), soil temperature at 10 cm depth (Onset Hobo S-TMB-M002; in moss in spruce stands, in mineral soil in birch stands), soil moisture at 10 cm depth (Onset Hobo S-SMD-M005; in moss in spruce stands, in mineral soil in birch stands), and photosynthetic active radiation at about 1.75 m above ground level (PAR, Onset Hobo S-LIA-M003). During each of the three growing seasons, air temperature was similar in both forest types, but was warmer in 2013 and 2015, and cooler in 2014 (Table 4.1). Soil temperatures were lower in spruce than in birch stands and remained relatively stable in 2013, 2014, and 2015 (Table 4.1). PAR was higher in spruce than in birch stands during the summer, as canopy cover was higher in birch stands (Table 4.1). I determined the canopy cover by analyzing hemispherical pictures taken ~30 cm above each moss patch sampled in 2014 (144 pictures) with Gap Light Analyzer (Frazer et al. 1999). Average soil moisture was similar between the two forest types, but much higher in 2014 than 2013 or 2015 (Table 4.1).

Table 4.2: Macroclimate variables in the study area for 2013-2015 in terms of accumulated growing degree-days, growing season precipitation, and average air temperature for the months of June, July, August and September. Data from the Fairbanks International Airport, Fairbanks, Alaska (USA) (Alaska Climate Research Center 2016).

	Sum growing degree-days (> 5°C)	Sum of precipitation (mm)	Average monthly air temperature (°C)			
			June	July	August	September
2013	1366.9	142.2	19.3	17.9	15.2	6.5
2014	1261.7	370.6	14.7	16.1	15.0	8.0
2015	1241.4	207.5	15.4	16.8	12.5	5.8

In 2014, I set up a 60 m transect in each birch or spruce forest in blocks A, B, and C in order to characterize vegetation. I conducted a visual estimation of percent ground cover of moss species, vascular plants, and leaf litter in six replicated 4 m^2 square plots equally spaced along the transect. The most common bryophytes were *H. splendens* and *P. schreberi* and they were more abundant in spruce than in birch stands (Table 4.1). Although no systematic attempt was made to quantify or identify species of cyanobacteria associated with these mosses, examination of a few samples revealed the presence of the cyanobateria genera *Nostoc* and *Anabaena*, both with heterocysts. Alder (*Alnus* spp.) and *Sphagnum* spp., which are also known to host N_2-fixing bacteria (Van Cleve et al. 1971, Basilier 1980), were present in some of the studied stands (Table 4.1). Deciduous leaf litter heavily dominated the forest floor in birch stands and was present in low abundance in spruce stands (Table 4.1). It was mostly composed of senesced birch leaves, but also included leaves from willows, alders, and ericaceous shrubs.

4.2.2 Sampling design for $^{15}N_2$ incubation assays

I conducted sampling for this study in 2013, 2014, and 2015, and the design used differed slightly every year as I learned about the system (see Table 4.3). Different moss patches were sampled across years, but the same patches were used within the seasonal 2014 sampling. In 2013, five monospecific *P. schreberi* or *H. splendens* moss patches of ~30 cm diameter were selected in the spruce and birch stands from blocks A and B. The moss shoots collected from the patches consisted of the green part of the moss (5 to 8 cm long). BNF rates were measured on July 27-28 2013 on 20 shoots of *P. schreberi* or 10 shoots of the larger *H. splendens* per moss patch. Pilot work was conducted in 2013 to determine which parts of the mosses were contributing the most to BNF and help me decide what to collect and analyze in the next years. For three out of the five samples only the side branches were used, for one sample both branches and stems were used, and for the last sample I separated upper (top 1.5 cm) and lower branches (below 1.5 cm) as well as stems for elemental mass spectrometer analysis (methods below). Branches and stems were dry weighed, which allowed an estimate of total moss BNF rates using ratios. I found no difference in BNF rates between upper and lower branches and between stems and branches in 2013 (see section 4.3.1 for results). Therefore, moss shoots were collected at a length of 5 cm from the apical meristem in 2014 and 2015 to get a standardized measurement of the upper green part of the moss mat. In 2014, I identified the closest monospecific moss patch to the 60 m transect used to characterize vegetation cover. Monospecific moss patches were

selected on both sides of the transect at 6 equally spaced location and were at least ~30 cm diameter (n = 12 patches per species per stand). BNF rates were measured on June 21-22, July 14-15, August 5-9, and September 26-27 2014 on 10 shoots of either *P. schreberi* or *H. splendens* per moss patch in the spruce and birch stand of block A. In August, I used the same methodology to select moss pacthes in blocks B and C to estimate spatial variation in BNF rates in relation to N availability. In 2015, BNF rates were measured on August 2-6 on 10 shoots of *P. schreberi* or *H. splendens* from five moss patches ~30 cm in each forest stand from blocks A and B (n = 5 patches per species per stand). These moss patches consisted in the procedural controls from the experiment conducted in Chapter 3 and were dominated by *H. splendens.* Data from *P. schreberi* in 2015 should be interpreted with caution as it was sampled from patches dominated by *H. splendens*.

BNF patches were watered with rainwater collected near Fairbanks 24 hours prior to, and immediately before the start of the incubation period to ensure a minimum water availability. In July 2014, I tested if watering the samples lead to an overestimation of potential fixation rates by incubating both dry and wet mosses. Moss samples to be enriched were inserted in a 60 ml translucent polycarbonate syringe. Each syringe was filled with 10 ml of $^{15}N_2$ (98% enriched, Cambridge Isotope Laboratories, Inc., U.S.A.) and another 10 ml of air. Sealed syringes were left *in situ*, i.e. laying on the moss patch of origin of the sample, for a 24 hour incubation period. After the incubation period, enriched and control samples were collected, weighed wet, oven-dried for 72 hours at 60°C, weighed dry, and finely ground. Nitrogen and carbon concentrations and atom% ^{15}N and ^{13}C values were determined using an Elemental Analyser coupled to a Continuous-flow Isotope Ratio Mass Spectrometer in the lab of Dr. Michelle Mack at either the University of Florida (Stable Isotope Mass Spec Lab in the Department of Geological Sciences, Finnigan-MAT DeltaPlus isotope ratio mass spectrometer Carlo Erba NA1500 Elemental Analyzer) or Northern Arizona University (Colorado Plateau Stable Isotope Lab, DELTA V Advantage mass spectrometer and Carlo Erba NC2100 Elemental Analyzer). The lab standards used for R (ratio of heavy to light stable isotope) were 0.003676 for N and 0.011237 for C. BNF was calculated by comparing the ^{15}N values from enriched and control samples (see Table A5.1 for detailed calculations). Raw data sets are archived online through the Bonanza creek LTER database (http://www.lter.uaf.edu/data/data-detail/id/669). To scale up my measurements of BNF into potential N fixed per unit area, five 10 cm diameter samples of each moss species were

excavated in each stand, and the number of green shoots, and wet and dry weight were calculated per unit area. In spruce stands, the depth of the green part of the moss carpet was about 2.2 cm ± 0.2 (SE); and 1.9 cm ± 0.2 (SE) in birch stands. This is shallower than the 5 cm length selected for the moss samples, because of the lateral growth form of pleurocarpous mosses. For moss shoots of a similar length, *P. schreberi* was about half to a third the weight of *H. splendens* (Table 4.1).

Table 4.3: Summary of the sampling design for inter-annual (August in 2013 to 2015), intra-annual (June to September 2014), and spatial (blocks in August 2014) variation in BNF rates.

Year	Date	Inter-annual variation	Intra-annual variation	Spatial variation	*n*[a]
2013	July 27-28	Blocks A, B	-	-	5
2014	June 21-22	-	Block A	-	12
	July 14-15	-	Block A	-	12
	August 5-9	Blocks A, B	Block A	Blocks A, B, C	12
	September 26-27	-	Block A	-	12
2015	August 2-6	Blocks A, B	-	-	5 (3-5 for *P. schreberi*)

[a] Number of sample replicates per moss species per forest type (spruce or birch) per block.

4.2.3 Data Analysis

In all of my analyses, BNF rates were log transformed to meet analysis of variance (ANOVA) assumptions of normality and homoscedasticity, and the R software was used for statistical calculations (R Core Team 2016).

4.2.3.1 Methodological tests of N_2-fixation measurements

I compared BNF rates in 2013 among upper and lower branches of the two moss species (n = 8 for each) and among branches and stems (n = 8 for each). Because of the small sample size, I pooled data for both species and forest types in blocks A and B for these two analyses. BNF rates of wet and dry samples in July 2014 (n = 24 for each) were also compared, using pooled data from both moss species and forest types (block A only). I used paired t-tests for unequal variances to conduct all three analyses.

4.2.3.2 Spatial and temporal variation:

I investigated inter-annual (late July or early August 2013, 2014 and 2015) variation in BNF rates among forest types, blocks (A and B), and moss species using a four-way ANOVA (year*forest*block*moss). Second, BNF rates were compared during the 2014 growing season (intra-annual variation) using data from the 12 moss patches of each moss species in the spruce and birch stand from block A in June, July, August and September. I used a mixed-effect model to account for the repeated measures taken on each moss patch using the library *lmerTest* (Kuznetsova et al. 2015). Spatial variation in BNF rates was assessed by comparing BNF by forest type and moss species among the three blocks using a three-way ANOVA with the data from August 2014.

4.2.3.3 Link with environmental variables

I used structural equation modelling (SEMs; Grace 2006) to estimate both direct and indirect effects of predictor environmental variables on observed BNF patterns. SEM allows the testing of a network of causal hypotheses based on my understanding of the ecological relationships among predictor variables and their influence on the BNF response variable (Grace 2006, Grace et al. 2012). Data from all years, months, and blocks were pooled in a single SEM and I used environmental covariates to account for shared variance associated with spatial and temporal pseudoreplication of the sampling scheme. Interpretation of the SEM results is therefore conservative, and used as a qualitative depiction of broad patterns and relationships

among variables. Creating a SEM that fits the data does not prove causal relationships beyond doubt, but it does establish probable causality (Grace et al. 2012). Thus, in this context SEMs are used to generate testable hypotheses for future mechanistic investigations of the environmental drivers of BNF (see discussion).

In my model, I included variables that varied according to year and month (average air temperature, average light availability and total precipitation 30 days prior to incubations), and a variable representing the effect of blocks (N availability), as well as categorical variables of forest type and moss species. Air temperature was selected rather than soil temperature, as it is more similar to the temperatures recorded inside the incubation syringes. Moreover, the surface of the moss layer experiences large diurnal variations in temperature (Rousk et al. 2014), which are more adequately captured by air temperature than by the cooler and more stable soil temperatures at 10 cm depth. My *a priori* model included all direct effects on BNF, as well as indirect effects of forest type on temperature, light availability and N mineralization, and correlations between light, temperature and precipitation (Figure 4.1). Air temperature and light availability were calculated from measurements collected at each of my six weather stations (each stand has one value per sampling period). Total precipitation was obtained from climate data recorded at the Fairbanks airport (Alaska Climate Research Center 2016). I represented variation in N availability among blocks using a single variable, N mineralization rates (Melvin et al. 2015). Forest type was included in my initial model as I am using it to account for potential unmeasured environmental variables associated with canopy type, as well as relationships between measured environmental variables and forest type.

Since SEMs assume linear relationships, the bivariate relationships were linearized by using the natural logarithm of BNF. Other relationships between variables are largely linear. All numerical variables were then standardized and I coded categorical variables as 0/1 (birch=0, spruce=1, and *H. splendens=0, P. schreberi*=1). I built my SEMs using the *lavaan* package in R (Rosseel 2012) with bootstrapping of 1000 iterations. Additional paths were added one at a time to my *a priori* model based on the highest modification indices, if they made ecological sense (Grace et al. 2012). I tested models with and without the forest type variable. Since both were adequate, I decided to use the more complex model, as it aligned more with my hypotheses. I determined if the SEM model was a good fit with my data by using a chi-square test ($p > 0.05$),

the root mean square error of approximation (RMSEA with lower 90% confidence intervals close to zero), and the comparative fit index (CFI > 0.90) (Grace et al. 2012).

4.2.3.4 Stand level fixation rates and N pools

Ecosystem N sources (inputs) and requirements in spruce and birch stands were estimated using published estimates from the same sites or similar forest stands in the region (Table 4.4, Table A5.2). Total N requirements were calculated by adding N requirements for annual net primary productivity (ANPP) of trees (foliage, wood and bark, and fine roots; (Ruess et al. 1996, Melvin et al. 2015)), and feather moss (Chapter 3), and subtracting N losses in plant litter (Melvin et al. 2015). Annual N requirements for trees and feather mosses were calculated as 32.63 kg N $ha^{-1}year^{-1}$ in spruce stands, and 80.04 kg N $ha^{-1}year^{-1}$ in birch stands. N sources were calculated by adding net N mineralization and nitrification (Melvin et al. 2015), BNF by vascular plants (Van Cleve et al. 1983b, Ruess et al. 1996), and atmospheric wet and dry N deposition (Jones et al. 2005). These N sources together account for 81% and 133% of N requirements in spruce and birch stands, respectively (Table 4.4). N loss estimates from the literature suggested that these values were likely to be low and highly site-specific. Estimates of total N losses summed to about 0.27 kg ha^{-1} $year^{-1}$ in spruce stands and 0.18 kg ha^{-1} $year^{-1}$ in birch stands, including N leaching (Van Cleve et al. 1983b), denitrification (Sponseller et al. 2016), and riverine exports of N (Jones et al. 2005).

I estimated the total amount of N_2 fixed by moss-cyanobacteria associations at the stand level using median N_2-fixation rates (μg N g dry $moss^{-1}$ day^{-1}), moss cover (%), moss shoot density (stems cm^{-2}), and moss weight per shoot (g dry moss $shoot^{-1}$). The median was used rather than the mean since the data distribution was skewed with large outliers. I estimated daily rates of BNF for June to September using measurements made in each month in 2014, and ratios of monthly rates for years when I had only one measurement during the summer (2013 and 2015). I estimated summer fixation rates for each measurement year in blocks A and B, and for all three blocks in 2014. These values were then summed to obtain a seasonal estimate of N_2-fixation and compared to the N budget to assess contributions of moss-associated BNF in boreal Alaska.

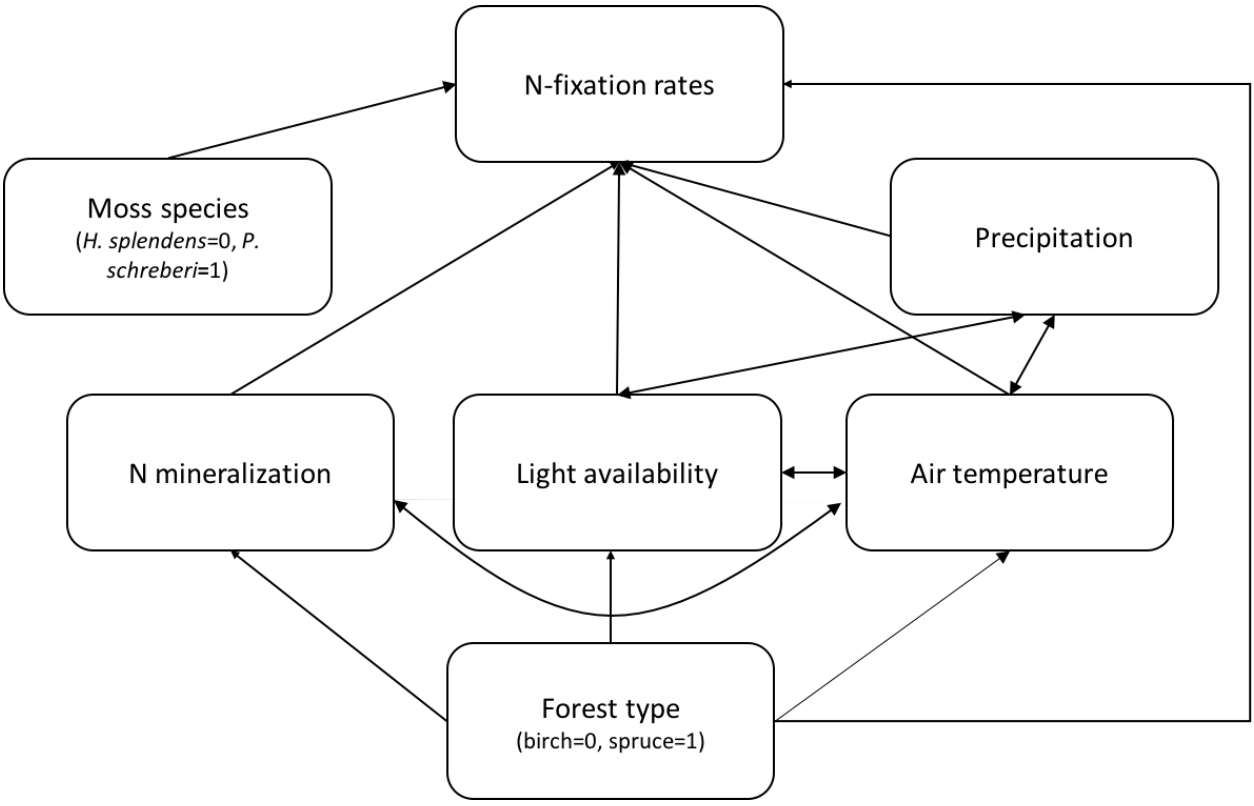

Figure 4.1: *A priori* pathways for structural equation model assessing drivers of N_2-fixation rates (log transformed values) using all measurements across forest types, moss species, years, months and blocks. Black lines with single arrows represent hypothesized regression pathways. Hypothesized covariances are indicated by double-headed arrows.

Table 4.4: Estimated N budget for upland black spruce and Alaska paper birch stands in interior Alaska. All values are given in kg N ha^{-1} $year^{-1}$. Detailed calculations are presented in Table A5.2. Footnotes indicate sources of data used in the table.

		Black spruce	Alaska paper birch
Plant N requirements	N allocation to tree growth (foliage, perennial wood and bark, fine roots, loss through litter)[a,b]	25.62	79.79
	N allocation to feather moss growth[c]	7.01	0.25
	Total vegetation N requirements	32.63	80.04
N Sources	N mineralization and nitrification[a]	21.46	91.82
	N_2-fixation vascular plants[b,d]	1.50	4.00
	Dry and wet N deposition[e,f,g]	0.30	0.30
	Total N Sources	26.50	106.85
	N sources / N requirement *100	81.21	133.49

[a] Melvin et al. (2015).
[b] Ruess et al. (1996).
[c] Chapter 3.
[d] Van Cleve et al. (1983b).
[e] Jones et al. (2005).
[f] Sponseller et al. (2016).
[g] Hinzman et al. (2006).

4.3 Results

4.3.1 Methodological tests of N_2-fixation measurements

Natural variation in the range of stable N isotope (^{15}N) was more or less constant across years, forest types, moss species and blocks ($\delta^{15}N$ = -2.05 ‰ ± 0.13 (mean ± SE, n = 71), range -3.81 to 2.26 ‰). Overall, natural $\delta^{15}N$ was similar among moss species and forest types, with values of -1.98 ‰ ± 0.18 (n = 35) for *H. splendens* and -2.14 ‰ ± 0.18 (n =36) for *P. schreberi*, and -1.90 ‰ ± 1.18 (n = 36) in birch stands and -2.23 ‰ ± 0.95 (n = 35) in spruce stands. This range of variation was much smaller than the values observed in the ^{15}N enriched samples ($\delta^{15}N$ = 1043.30 ‰ ± 51.51 (n = 362), range -4.15 to 7,561.17 ‰).

No difference was found in fixation rates between upper and lower branches in *P. schreberi* and *H. splendens* in spruce and birch stands in 2013 (paired t test: $F_{1,7} = 0.0393$, $p = 0.8484$) or between main stems and branches (paired t test: $F_{1,7} = 0.0056$, $p = 0.9427$). Average moss moisture was 40.6% ± 5.4 in the non-watered mosses, while it was 76.9% ± 3.8 (SE) in the watered treatment. I found no significant difference between fixation rates of watered (68.4 µg N/g dry moss/day ± 6.5) and non-watered mosses (60.0 µg N/g dry moss/day ± 6.3) for both forest types and moss species (paired t test: $F_{1,23} = -1.736$, $p = 0.096$), indicating that the watering method should have a small influence on the rates measured.

4.3.2 Inter-annual variation (2013-2015)

Estimates of BNF per unit moss biomass in boreal feather mosses showed significant variation among years, stand types, moss species, and study blocks. Year was the dominant driver of the variation, explaining about 52% of the total variation in N_2-fixation rates by itself (Figure 4.2; Table A5.3a). Inter-annual variation followed variation in precipitation, while air temperature remained similar among the three years (Table 4.1; Table 4.2). BNF rates were the highest in 2014 ($\bar{x}$ = 79.9 µg N fixed g dry moss^{-1} day^{-1}), which was the rainiest year, and higher in 2013 ($\bar{x}$ = 15.4 µg N fixed g dry moss^{-1} day^{-1}) than in 2015 ($\bar{x}$ = 1.7 µg N fixed g dry moss^{-1} day^{-1}). Light availability was higher in 2013 than in 2014 or 2015 (Table 4.1), and wildfire smoke was reported in the Fairbanks area during the 30 days prior to sampling for 8 days in 2013, 0 day in 2014, and 9.5 days in 2015.

BNF rates per unit of moss biomass measured in late July-early August were significantly higher in spruce stands than in birch stands in 2013 and 2014, but not in 2015 (Table A5.3a).

Comparison of BNF rates between moss species also differed among years (Table A5.3a), with BNF rates being higher in *H. splendens* in 2013 and 2015, and higher in *P. schreberi* in 2014. BNF rates were higher in the spruce stand of block A than in the spruce stand of block B, but higher in spruce than in birch stands in both blocks (Figure 4.2; Table A5.3a).

4.3.3 Intra-annual variation (June-September)

Rates of BNF per unit moss biomass changed significantly throughout the growing season (Figure 4.3; Table A5.3b). As for inter-annual variation, BNF rates followed weather trends. Air temperatures in 2014 were the highest in July, lower in September, and similar in August and June (Table 4.2). June received about 90 mm of rain, July 147 mm, August 58 mm, and September 73 mm. July 2014 was the warmest and wettest month of the study period (Alaska Climate Research Center 2016). For both moss species and both forest types, BNF rates increased from June to August, then declined in September. BNF rates remained higher for *P. schreberi* than *H. splendens* throughout the growing season, and higher in spruce than in birch stands (Table A5.3b). *Pleurozium schreberi* seemed to have a BNF peak in July in birch stands, while BNF of *H. splendens* in birch stands remained low throughout the growing season with a peak in August. In spruce stands, BNF rates peaked only in August, with July and September also having fairly high rates. BNF rates averaged across the 2014 measurements were the lowest for *H. splendens* in birch stands, similar between *P. schreberi* in birch stands and *H. splendens* in spruce stands, and the highest for *P. schreberi* in spruce stands (Figure 4.3; Table A5.3b).

4.3.4 Spatial variation in N_2-fixation (blocks)

BNF rates differed among moss species, with rates in *P. schreberi* higher than those of *H. splendens* across all blocks and forest types ($F_{1,132} = 25.04$, $p < 0.0001$; Figure 4.4; Table A5.3c). Fixation rates were overall higher in spruce stands than in birch stands ($F_{1,132} = 27.55$, $p < 0.0001$), however this difference was only found in blocks A and B ($F_{1,132} = 12.03$, $p < 0.0001$). In birch stands, fixation rates were lowest in block A and highest in block C, while in spruce stands, fixation rates were highest in block A and lowest in block C (Figure 4.4; Table A5.3c). This pattern was similar to that of N availability, which was higher in birch than in spruce stands, and higher in block C than in blocks A and B (Table 4.1).

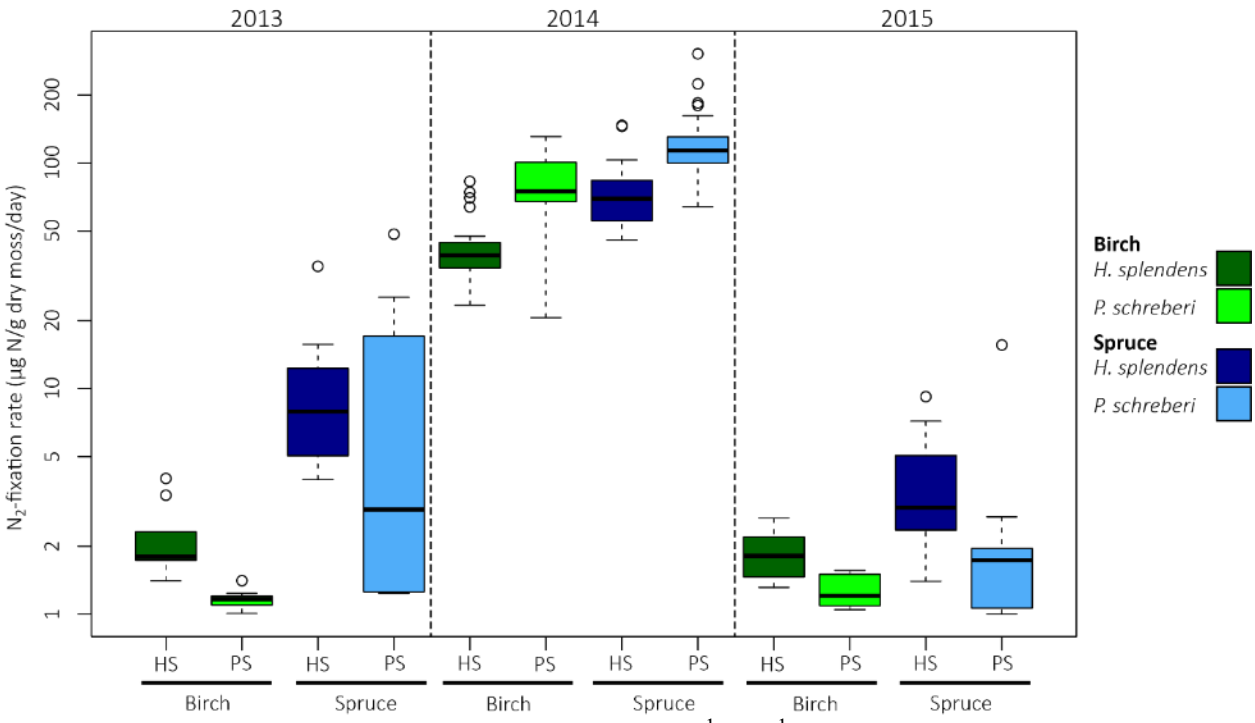

Figure 4.2: Dinitrogen fixation rates (µg N g dry moss^{-1} day^{-1}) of *Hylocomium splendens* (HS; darker colours) and *Pleurozium schreberi* (PS, lighter colours) in birch (green) and spruce (blue) stands from blocks A and B (pooled) in late July-early August 2013 (n = 10, 5 per species per forest type per block), 2014 (n = 24, 12 per species per forest type per block), and 2015 (n = 10, 5 per species per forest type per block). Medians are indicated by the black horizontal lines, boxes include 25–75% quantiles of the data, while whiskers include 5–95%. Outliers are shown as open circles. Post-hoc Tukey HSD tests (α = 0.05) indicate that fixation rates were highest in 2014, and higher in 2013 than 2015; higher in spruce than in birch stands in 2013 and 2014 but not 2015; and higher in *P. schreberi* than *H. splendens* in 2014, with the reverse pattern (*H. splendens* > *P. schreberi*) in 2013 and 2015. Note the log scale of the y-axis.

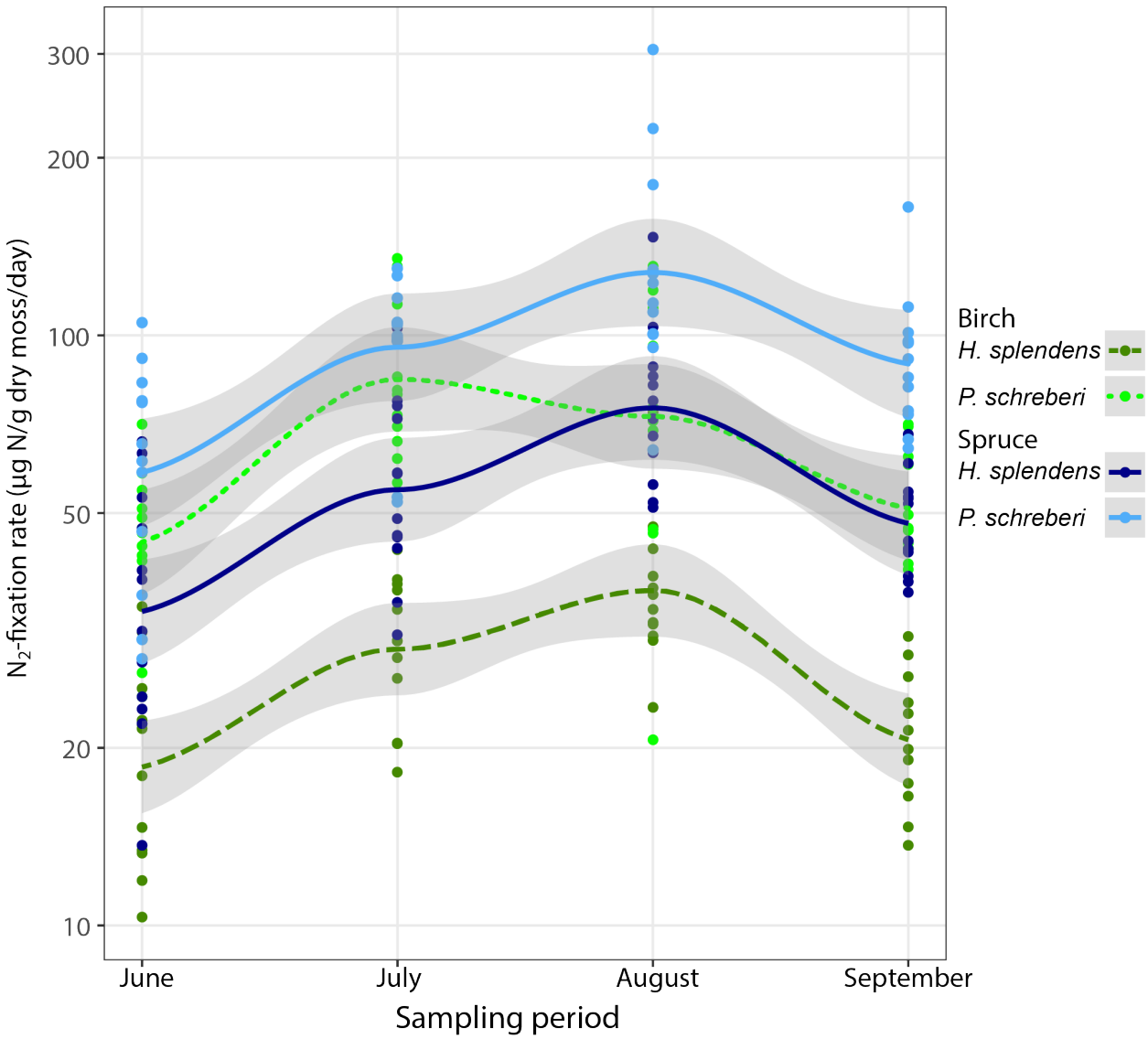

Figure 4.3: Fixation rates (µg N g dry moss^{-1} day^{-1}) for *Hylocomium splendens* (dark colours) and *Pleurozium schreberi* (light colours) in spruce (blue, solid) and birch (green, dashed) stands in June, July, August and September 2014 in block A. Each point represents the fixation rate of one sample. Curves and shadings represent a LOESS (locally weighted scatterplot smoothing) curves with a smoothing parameter of 1 for each series of points and 95% confidence interval. Note the log scale of the *y*-axis.

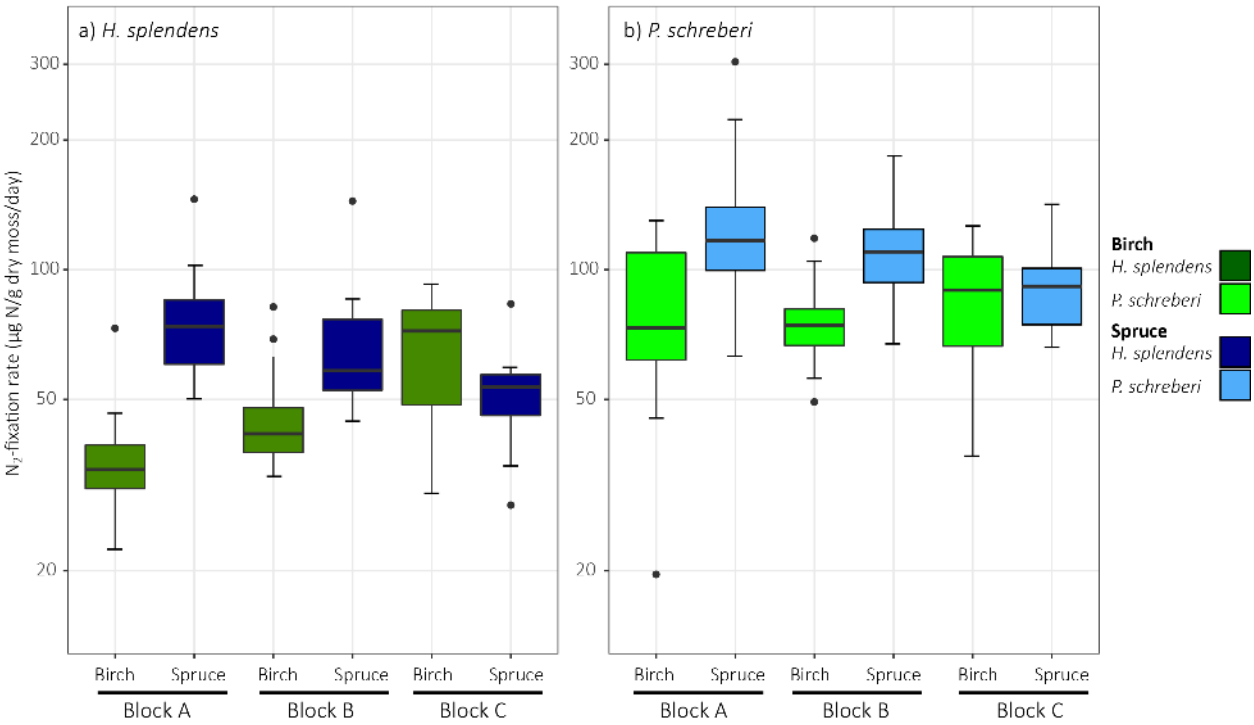

Figure 4.4: Fixation rates (µg N g dry moss^{-1} day^{-1}) for *Hylocomium splendens* and *Pleurozium schreberi* measured in August 2014 in birch (green) and spruce (blue) stands in the 3 spatial blocks. Medians are indicated by the black horizontal lines, boxes include 25–75% quantiles of the data, while whiskers include 5–95%. Outliers are shown as dots. Note the log scale of the *y*-axis.

4.3.5 Relation to environmental variables

In the final SEM model (Figure 4.5; Table A5.4), the direct effects of moss species and forest types were in accordance with the previous analyses, showing higher rates of BNF in spruce than in birch stands, and higher rates in *P. schreberi* than *H. splendens* (likely due to the larger sampling effort in the year when the former had much higher rates of BNF). BNF was positively associated with higher precipitation in the month leading to the measurements, and negatively impacted by higher temperatures and light as those two variables were strongly correlated. Light availability was higher in spruce than in birch stands, but no difference in air temperature was found among stand types (Table 4.2). Forest type had a strong effect on N mineralization rates, with much lower rates in spruce stands. I expected that N mineralization rates would be inversely related to BNF. However, once accounting for the indirect effect of forest type, I found that BNF was positively associated with N mineralization rates (Figure 4.5, Table A5.4). My model suggests that the indirect impact of forest type on BNF rate may be through its effect on N mineralization and light, rather than through changes air temperature. Moreover, the strong direct path between forest type and BNF rates indicates that other stand associated variables that were not measured may be important in driving rates of BNF (e.g., moss moisture regime; Figure 4.5).

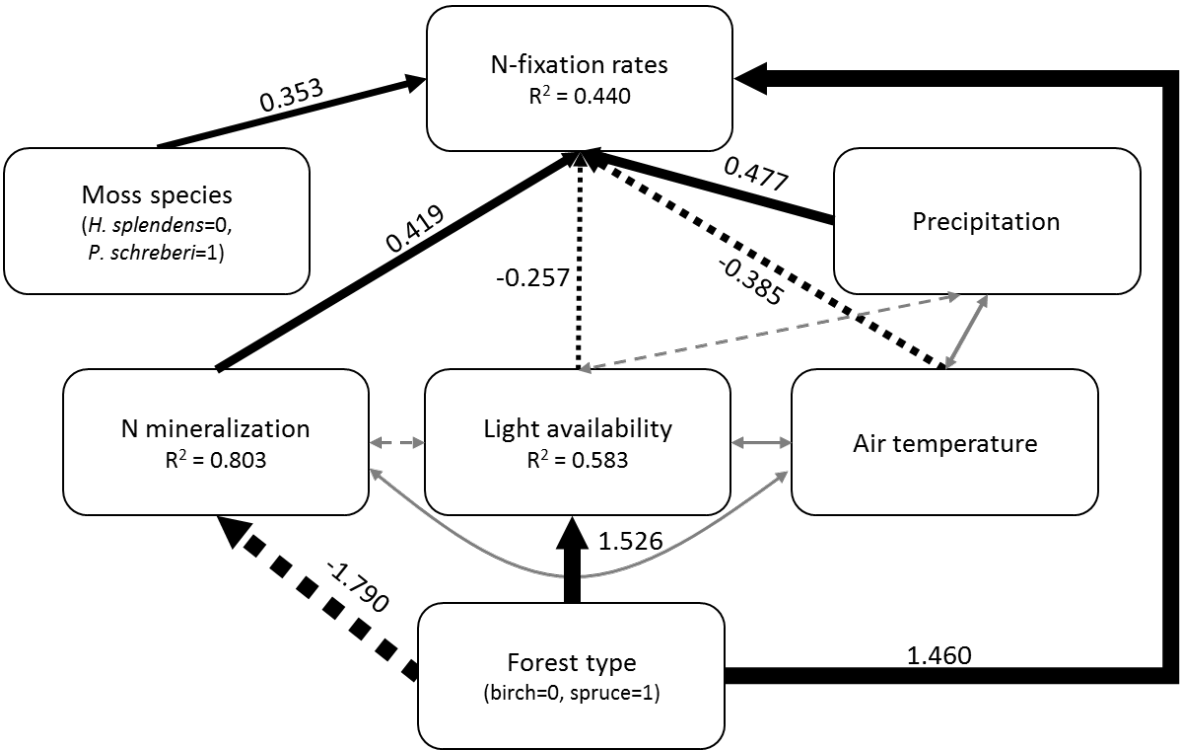

Figure 4.5: Structural equation model pathways assessing drivers of N_2-fixation rates (log transformed values, µg N g dry moss^{-1} day^{-1}) using all measurements across forest types, moss species, years, months and blocks ($p(\chi 2) = 0.232$, root mean square error of approximation (RMSEA) 90% confidence interval = 0.00 - 0.079, comparative fit index (CFI) = 0.998). Black lines represent significant regression pathways. Solid lines represent positive pathways, while dashed lines are negative pathways. Significant covariances are indicated by grey double-headed arrows and their coefficients are found in Table A5.4. Path coefficients are the standardized prediction coefficients from the SEM model with the width of the arrow indicating the magnitude. R^2 values are included for all significant endogenous variable pathways. Sample size is 363, with unequal numbers of samples for the different years, months and blocks (see sampling design).

4.3.6 BNF contribution to N budgets

Total N_2 fixed per unit ground area, as well as inter-annual variation were much larger in spruce than in birch stands, where BNF rates and moss cover were always low (Table 4.5). In the drier 2013 and 2015 years, which had lower rates of BNF and lower total amounts of N_2 fixed, cyanobacteria communities associated with *H. splendens* were the major N_2-fixers, while in the wet 2014 year, communities associated with *P. schreberi* fixed more N_2. Rates of BNF and total amount of N_2 fixed in 2014 were surprisingly high and orders of magnitude larger than the other two years. The range of variation among blocks for each moss species in each forest type in 2014 was smaller (2-10 fold, Table 4.6) than that of the inter-annual variation (46-317 fold). Even in cases like block C where daily BNF rates were similar in birch and spruce stands (Figure 4.3), the total N_2 fixed per unit area was still a lot larger in spruce than in birch stands (Table 4.6). The highest ecosystem N input from moss-cyanobacteria associations was found in the spruce stand from block A, which had the highest BNF rates, as well as the highest moss cover, shoot density, and dry weight of moss per unit area (Table 4.6, Table A5.2).

I compared the amount of N_2 fixed per unit area to feather moss N requirements per unit area and found a wide inter-annual variation, with values ranging from 2-200% in birch stands, and 11-429% in spruce stands (Table 4.5). BNF in birch stands accounted between 0.01 and 1% of the total annual N requirements in birch stands, while they accounted for 2 to 92% in spruce stands (Table 4.5). In spruce stands, N inputs from moss-cyanobacteria associations have the potential to be larger than combined BNF from vascular plants and dry and wet N deposition in a 'typical' year (e.g. 2013 and 2015), and even larger than mineralization and nitrification (Table 4.4) in the spruce stand of block A in exceptional years like 2014. Spatial variation in the amount of N_2 fixed per year was measured during 2014, which was a year with exceptionally high rates of fixation, and I found that N fixed by both moss species in spruce stands and in birch stands A and B almost equaled or exceeded feather moss annual N requirements (Table 4.6). Nevertheless, the low abundance of mosses in deciduous stands meant that the contribution of moss BNF to N budget was negligible in all three blocks in birch stands. The contribution of N fixed in association with both species of feather mosses to N budgets was very variable in spruce stands, ranging from 13 to 44% of the ecosystem N requirements (Table 4.6).

Table 4.5: Moss biomass (top 5 cm) and estimated average fixed N (kg N ha^{-1} $summer^{-1}$) over the 2013, 2014, and 2015 summers (June-September). Total fixation rates were obtained by using moss cover, density, dry weight, and median fixation rates for each month. 2013 and 2015 monthly data were estimated based on the 2014 ratios. The values presented are the averages of blocks A and B.

Forest type	Moss species	Moss cover (%)	Moss dry weight (g m^{-2})	2013 N_2-fixation rate (kg N ha^{-1} $summer^{-1}$)	2014 N_2-fixation rate (kg N ha^{-1} $summer^{-1}$)	2015 N_2-fixation rate (kg N ha^{-1} $summer^{-1}$)
Birch	*H. splendens*	3.0	7.0	0.005	0.185	0.004
	P. schreberi	1.6	4.2	0.001	0.317	0.001
		% feather moss requirements [a]		**2.39**	**199.74**	**1.99**
		% Total N requirements [a]		**0.01**	**0.63**	**0.01**
Spruce	*H. splendens*	69.4	224.3	1.975	14.577	0.710
	P. schreberi	38.1	137.4	1.424	15.482	0.086
		% of feather moss requirements [a]		**48.48**	**428.71**	**11.35**
		% Total N requirements [a]		**10.42**	**92.12**	**2.44**

[a] Calculated from the N budget presented in Table 4.4.

Table 4.6: Estimates of potential fixed N (kg N ha^{-1} $summer^{-1}$) in 2014 for all blocks, forest types and moss species based on median N_2 fixation rates.

Forest type	Moss species	Block	Moss cover (%)	Moss dry weight (g m^{-2})	Median August fixation rate (µg N/g dry moss)	N fixed (kg N ha^{-1} $summer^{-1}$)	% feather moss N requirements	% vegetation N requirements
Birch	*H. splendens*	A	1.8	5.3	34.363	0.167	**66.45**	**0.21**
		B	4.1	7.6	41.594 [a]	0.202	**80.37**	**0.25**
		C	0.1	0.3	72.156 [a]	0.021	**8.36**	**0.03**
	P. schreberi	A	1.6	4.9	73.240	0.365	**145.23**	**0.46**
		B	1.5	3.6	74.351 [a]	0.270	**107.43**	**0.34**
		C	0.6	2.4	90.023 [a]	0.216	**85.95**	**0.27**
Spruce	*H. splendens*	A	87.6	362.3	73.889	23.061	**328.90**	**70.68**
		B	51.2	121.4	58.292 [a]	6.094	**86.91**	**18.68**
		C	45.6	118.2	53.292 [a]	5.429	**77.43**	**16.64**
	P. schreberi	A	42.6	182.5	117.023	20.526	**292.75**	**62.91**
		B	33.7	98.7	110.057 [a]	10.438	**148.87**	**31.99**
		C	37.3	94.9	91.501 [a]	8.340	**118.95**	**25.56**

[a] Rates for June, July and September in blocks B and C were estimated using the ratio between months in block A.

4.4 Discussion

BNF by feather moss-cyanobacteria associations has the potential to be a source of N equal or larger than all other N sources combined in the mature black spruce stands of this study. BNF rates were generally higher in black spruce than in Alaska paper birch stands for both moss species, although they were low in all stand types in 2015. Environmental factors specific to each forest types are likely important drivers of variation in BNF rates by feather moss-cyanobacteria associations. Boreal landscapes dominated by deciduous tree species, as well as areas that may be converted from coniferous to deciduous stands following increased fire severity in Alaska (Johnstone et al. 2010, Mann et al. 2012), should present lower rates of BNF and a lower contribution of BNF to ecosystem functioning. Additionally, I highlighted the critical importance of stochastic factors associated to weather patterns in driving BNF. Indeed, year and month of sampling had large impacts on fixation rates that could override canopy, moss species, and micro-environmental variables. My results support the idea that N inputs from feather moss-cyanobacteria associations contribute significantly to N pools in interior Alaska, particularly in black spruce stands where feather mosses are abundant, while their contribution is likely negligible in deciduous birch stands.

Boreal forests have large stocks of organic N in their soils (Van Cleve and Alexander 1981), yet plant productivity is often limited by N turnover and access by plants and microbial symbionts (Tamm 1991, Vitousek and Howarth 1991, Cleveland et al. 1999). Until recently it was thought that boreal forests lacked widespread N_2-fixers (Tamm 1991, Cleveland et al. 1999). The amount of N_2 fixed by feather mosses in upland spruce stands was extremely variable among years, and could contribute up to 92.1% of the total vegetation (trees and mosses) N requirements in 2014, 10.4% in 2013 and 2.4% in 2015. Feather moss contribution to the N budget in birch stands was relatively minor, ranging between 0.01 and 0.63%. In both forest types, the contribution of *H. splendens* was higher in 2013 and 2015, while *P. schreberi* contributed more ecosystem N in 2014. However, the average contribution of the two moss species over the three years were similar, on the order of about 5.7 kg N ha^{-1} $year^{-1}$. A N input threshold of 10.25-12.25 kg N ha^{-1} $year^{-1}$ has been suggested, over which moss associated BNF decreases (Zackrisson et al. 2004, Ackermann 2013), which is far below N inputs in birch stands (Table 4.4, Table A5.2). BNF contributions by feather mosses have been reported to range

between 0.01-7 kg N ha^{-1} $year^{-1}$r in boreal, mostly coniferous, stands (Lindo et al. 2013). The rates measured during the wet year of 2014 in spruce stands clearly exceed this range (~15 kg N ha^{-1} $year^{-1}$ for each moss species, and from 14-44 kg N ha^{-1} $year^{-1}$ depending on the block). The exceptional amount of N_2 fixed by feather mosses in 2014 exceeded feather moss N requirements. It is still unclear what happens to the fixed N_2 and how much of it is trapped in recalcitrant moss litter, transferred to other ecosystem components through mycorrhizal fungi (Carleton and Read 1991, Lagerström et al. 2007), or leached following drying and rewetting events (Carleton and Read 1991, Rousk et al. 2014). To my knowledge, there has been little work investigating such a process to date and it may be an interesting avenue, especially given the enormous inter-annual variation found.

Years were the largest source of variation in BNF rates, with rates in 2014 being 9-fold higher than in 2013, and 38-fold higher than in 2015. This pattern of inter-annual variation was similar to that of the precipitation, with 2014 receiving twice the amount of rain of 2013 and 2015, or twice the Fairbanks long-term average (Hinzman et al. 2005). Peaks in BNF have been reported to occur in June and September in Scandinavia (Zackrisson et al. 2004), periods where drought stress and photoinihibition are expected to be lower (Sveinbjörnsson and Oechel 1992, DeLuca et al. 2002, Zackrisson et al. 2004), or in the middle of the growing season in the Arctic (Alexander and Schell 1973, Chapin et al. 1991). BNF rates varied throughout the growing season within 2014, with a peak in fixation rate occurring in late-July and early August for both moss species and in both forest types. Moss moisture was high (70-95%) in July and August 2014 and may not have been a limiting factor for BNF. The range of variation in BNF rates of *H. splendens* and *P. schreberi* in black spruce and Alaska paper birch stands between blocks was smaller than the inter- and intra-annual variation. Fixation rates were higher in spruce stands in block A and to a lesser extent in block B, but both forest types had similar BNF rates in block C. This difference in the relative importance of the components of variation investigated gives us insights into the potential relative importance of environmental drivers of BNF, with factors that vary on a yearly scale seemingly more important than factors that vary in space within each forest type.

My study was not designed to specifically test the environmental mechanisms driving BNF patterns, but some interesting relationships emerged with temperature, light availability, N availability, and precipitation. Numerous studies have found a positive impact of temperature on

BNF by free-living and moss-associated cyanobacteria (Chapin et al. 1991, Solheim and Zielke 2002, Zielke et al. 2002, Gundale et al. 2012a). Conversely, I found that temperature within the narrow range of means observed (12-16 °C) had a negative association with BNF rates, possibly because of a decrease in ambient moisture with increased temperatures (Gundale et al. 2009, Gundale et al. 2012a, Gundale et al. 2012b). BNF has been suggested to be dependent on light availability and moss photosynthesis (Gundale et al. 2012b, Sorensen et al. 2012), but my data did not strongly support this idea. Light availability was relatively high in all cases and may not have reached a limiting level; furthermore, in my field-based measurements the effect of light was confounded with that of temperature and moisture. BNF is an energetically expensive process and rates of BNF as well as moss colonization by cyanobacteria are known to decrease under high N availability (Zackrisson et al. 2004, DeLuca et al. 2008, Gundale et al. 2011). Differences in N cycling and availability between the two forest types may explain the higher BNF rates found in spruce stands (Table 4.4). Contrary to my expectations, once I accounted for the strong effect of forest type on N mineralization rates, N availability was positively associated with BNF. I suppose that this may be due to the sampling design that emphasized variation among year in blocks A and B over variation among blocks. Mineralization rates were much higher in the spruce stand of block C, which had the lowest BNF and is in accordance with my expectations of a negative impact of N mineralization (availability) on BNF rates. Natural variability in N among sites may not be large enough to really induce major changes in BNF, such as those created by artificial N additions. Other factors that may affect N_2-fixation rates were not measured, such as abundance and colonisation of mosses by cyanobacteria (Whiteley and Gonzalez 2016), molybdenum or iron availability (van Groenigen et al. 2006, Rousk et al. 2016), or trophic interactions within the bryosphere (Kardol et al. 2016).

Sufficient moisture is necessary for critical processes in mosses and cyanobacteria such as photosynthesis and BNF (Zielke et al. 2002, Zielke et al. 2005, Gundale et al. 2009, Jackson et al. 2011, Gundale et al. 2012b). Solheim et al. (2002) have found that *H. splendens* BNF rates increased with increased precipitation. Indeed, BNF was positively associated to precipitation, and I found that both inter- and intra-annual patterns of fixation rates resembled precipitation patterns. I found that BNF associated with *P. schreberi* seemed to be more sensitive to the lack of precipitation than *H. splendens*. This was surprising given that BNF by cyanobacteria associated with *P. schreberi* was thought to be more drought resistant than that of *H. splendens*

(Zackrisson et al. 2009, Gundale et al. 2012a). *Pleurozium schreberi* is the dominant moss species both in cover and BNF in most stands where BNF has been investigated in Scandinavia (DeLuca et al. 2002, Gundale et al. 2009, Zackrisson et al. 2009). Interior Alaska receives much less precipitation (Hinzman et al. 2005) than most areas of Scandinavia where moss BNF has been studied (e.g. Lagerström et al. 2007). This apparent capacity of interior Alaska's *H. splendens* N_2-fixing microbial communities to thrive under dry conditions may in part explain their dominance at my site. Precipitation, or other sources of moisture such as snow melt, is likely the most critical limiting factor driving patterns of BNF in this part of the boreal forest.

4.5 Conclusion

BNF by feather moss-cyanobacteria associations appears to be a significant contributor to N cycling in interior Alaska's black spruce forests, especially where nutrient availability is limited and when precipitation is high, while it is likely to be a negligible part of the N cycle in birch forests. The extent of the contribution to N in spruce stands was strongly dependent on the year of sampling, and varied significantly with annual variations in precipitation. Moss species, canopy type, seasonal variation, and nutrient availability all influenced BNF rates in interior Alaska's boreal forest. Fixation rates were higher in spruce stands, in August, and where N availability was low. In birch stands, fixation rates remained low through the summer and across the nutrient gradient (potentially mineralizable N). Daily fixation rates per mass of moss were higher for *P. schreberi* than for *H. splendens* in 2014, but on average both moss species contributed similarly to BNF, which was unexpected. My study also highlights the complexity of interactions among some of the environmental factors affecting BNF. The interactions of all the factors create challenges for predicting responses to individual environmental factors, such as temperature. Nevertheless, my results point towards the importance of precipitation and moisture as controls over temporal variation in BNF in dry environments such as interior Alaska. In summary, this study highlighted the low contribution of BNF in Alaskan deciduous forests, as well as large inter-annual variation in BNF in spruce stands driven by climate. In this context, changes in climate (e.g. change in precipitation regime), fire regime, or switches in canopy dominance in the boreal forest are likely to significantly alter the dynamics of BNF by moss-cyanobacteria associations. For example, increases in summer precipitation are likely to increase

BNF rates and contributions to N budgets, while increases in deciduousness in the landscape induced by increased fire severity (Mann et al. 2012) are likely to reduce landscape-scale contribution of BNF to N cycling.

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CHAPTER 5: CONCLUSIONS

5.1 Summary of rationale and research objectives

Bryophytes, a group of nonvascular plants comprising mosses, liverworts, and hornworts, are considered as keystone taxa in many ecosystems (Rochefort 2000, Frego 2007, Turetsky et al. 2010), as they often carry out ecosystem processes of an importance disproportionate to their fraction of biomass (Sveinbjörnsson and Oechel 1992). With their position at the interface between above and belowground processes, bryophytes are indeed well positioned to play critical roles in plant-soil feedbacks (Lindo et al. 2013). Bryophytes are ubiquitous components of the understory in coniferous boreal forests throughout the circumpolar region (Turetsky et al. 2012). There, they contribute to aboveground productivity and carbon (C) storage, nutrient cycling, soil temperature regulation and moisture retention, interact with vascular plants (Turetsky 2003, Cornelissen et al. 2007, Turetsky et al. 2012), and host dinitrogen (N_2)-fixing cyanobacteria (DeLuca et al. 2002, DeLuca et al. 2007). In coniferous boreal forests dominated by black spruce (*Picea mariana* (Mill.) B.S.P.), extensive moss carpets composed of pleurocarpous (feather) mosses such as *Hylocomium splendens* (Hewd) Schimp., *Pleurozium schreberi* (Brid.) Mitt., and *Ptilium crista-castrensis* (Hewd.) De Not. dominate the forest floor and produce up to 20 to 40% of total net primary production (Bond-Lamberty et al. 2004, Turetsky et al. 2010).

While mosses are abundant in coniferous black spruce forests, broadleaf deciduous stands do no support abundant moss cover (Hart and Chen 2006, Turetsky et al. 2010). In black spruce stands, the high moss net primary production and accumulation of thick organic layers promote further dominance of black spruce (Fenton et al. 2005, Hollingsworth et al. 2006, Johnstone et al. 2010a). Reduced moss cover in deciduous stands leads to warm and dry soils, high nutrient availability, and shallow organic layers, all of which are conditions that are favourable to the stability of this canopy type (Johnstone et al. 2010a). There is still considerable uncertainty with regards to the mechanisms driving this discrepancy in moss abundance between deciduous and coniferous stands. Observations suggest that broadleaf litter prevents moss establishment and growth through the formation of a physical barrier to moss growth (Van Cleve et al. 1983b, Beatty and Sholes 1988, Startsev et al. 2008), altered soil chemical conditions by increasing nutrient availability (Messier et al. 1998, Côté et al. 2000, Turetsky et al. 2012), and allelopathic effects (Légaré et al. 2005, Startsev et al. 2008).

Functional traits, the characteristics that define the roles of species in ecosystems, of canopy trees, such as abundant broadleaf litter production in deciduous stands vs. low needle litter production in spruce stands, impact bryophyte abundance and community composition. These impacts are hypothesized to be critical in stabilizing alternate deciduous and coniferous states in boreal Alaska. The purpose of my research was therefore to measure the respective impacts of canopy type and deciduous broadleaf litter on bryophyte post-fire succession, and on their roles in the C (biomass) and N cycles in boreal deciduous and coniferous stands. First, I used a 163 years chronosequence to assess temporal changes in bryophyte abundance and species composition in deciduous (trembling aspen and Alaska paper birch) and coniferous (black spruce) stands in interior Alaska (Chapter 2). I then used a field-based experiment (Chapter 3) using feather moss transplants to unravel the respective impacts of canopy type and leaf litter inputs as mechanisms driving moss growth and abundance in deciduous (low moss cover) and coniferous (high moss cover) forests. Lastly, in Chapter 4, I used stable isotope ($^{15}N_2$) enrichments to measure how moss-associated N_2-fixation varied according to stand type (coniferous vs. deciduous), as well as to feather moss species (*H. splendens* vs. *P. schreberi*), and across space and time.

5.2 Divergent successional trajectories of bryophyte communities in deciduous vs. coniferous stands

In this study, I examined if differences in moss communities between mature coniferous and broadleaf deciduous stands arised due to environmental differences or are driven by plant-plant interactions associated with differences in broadleaf leaf litter (biotic interactions). Boreal forests are characterized by large stand-replacing fires (Johnson 1992, Payette 1992), which trigger forest regeneration and successional changes in bryophyte communities (Foster 1985, Turetsky et al. 2010). Specifically, I investigated if bryophyte communities in different stand types followed distinct or divergent successional trajectories following fire, which gave insights in the relative importance of post-fire legacies, environmental conditions, and plant-plant interactions through leaf litter inputs.

In the first years following fire, bryophyte communities and cover (75%) were similar in deciduous and coniferous stands types, being initially dominated by colonizing and pyrophilitic

species such as *Ceratodon purpureus* (Hedw.) Brid., *Leptobryum pyriforme* (Hedw.) Wilson, *Marchantia polymorpha L.,* and later on by *Polytrichum* spp. At about 20 years after fire, the early colonizing species started to decline in abundance in all stand types, possibly because of a decline in light availability as canopies start to mature. Bryophyte abundance and species composition diverged between coniferous and deciduous stands around 40 years after fire. At this point in succession, bryophyte abundance collapsed in maturing deciduous stands, which I attributed to canopy closure and reaching a threshold in leaf litter cover of 75%. That threshold was never met in maturing coniferous stands and relay succession of bryophyte species towards communities dominated by feather mosses took place. Leaf litter cover was also inversely related to lichen cover, suggesting that leaf litter has the potential to affect not only bryophytes but also lichen communities. In black spruce stands, colonizing species were gradually replaced by species such as feather mosses like *H. splendens*, *P. schreberi*, and *Tomentypnum nitens* (Hedw.) Loeske, and by acrocarpous mosses like *Aulacomnium palustre* (Hedw.) Schwägr. Interestingly, the increase in the cover of *H. splendens* seemed mostly related to time since fire, while the increase in cover of *P. schreberi* was closely associated to higher black spruce basal area. The re-establishment of feather mosses over time in black spruce stands lead to deeper organic layers, lower pH, and higher soil moisture. *Sphagnum* spp. started to establish in some of the oldest and wettest spruce stands sampled and was strongly associated to deeper organic layers. It has been suggested that *Sphagnum* spp. establishment is due to facilitation by feather mosses raising the water table (Fenton and Bergeron 2006), or declines in canopy cover increasing light availability as stands age, possibly insect-induced (Hollingsworth et al. 2010, Turetsky et al. 2010). With a relatively short fire return interval of 100 to 150 years (Yarie 1981, Van Cleve et al. 1983a), it is unlikely that upland black spruce stands in interior Alaska will undergo as much paludification as is the case in eastern boreal forests (Fenton et al. 2005, Fenton and Bergeron 2006).

Bryophyte communities in upland interior Alaska followed divergent successional trajectories in broadleaf deciduous (aspen or birch dominated) vs. coniferous (black spruce dominated) forests. This divergence suggests that traits associated with canopy-forming trees, especially the production of broadleaf litter, play important roles in driving succession in bryophyte communities. Evaluating the mechanisms of succession in black spruce stands (competition vs. facilitation hypotheses) could be done using moss transplants across different forest types and successional stages paired with measurements of spore rain and seeding

experiments. Nevertheless, results from this observational study provided empirical evidence about temporal trends of bryophyte succession under different boreal canopy types and support leaf litter as a potential key driver of moss abundance in forested ecosystems.

5.3 Environmental controls on bryophyte abundance among stand types and ecosystem impacts

Mosses are dominant components of the boreal forest understory and their presence or absence is key in boreal C and N cycling (Turetsky 2003, Lindo et al. 2013). Overall, indiviual moss shoots grew slightly better in spruce than in birch stands. However, average productivity of *H. splendens* was low in birch stands (~ 0.8 g m^{-2} $year^{-1}$; < 1%), while it was a significant contributor to stand productivity in black spruce stands (~ 54.2 g m^{-2} $year^{-1}$; 22%). Feather mosses and *Sphagnum* spp. tend to produce large amounts of slow-decomposing biomass, which promote soil microclimate regulation and permafrost stability (Jorgenson et al. 2010). This could be attributed to higher soil moisture, lower pH, and higher light availability in the studied spruce stand, all of which should favour moss growth, and are in turn promoted by moss functional traits (Fenton and Bergeron 2006, Turetsky et al. 2012) and by the low evapotranspiration rate of black spruce (Bonan 1991). This highlights the strong feedbacks between mosses and black spruce forests. The low bulk density of feather moss mats also make them an important combustion fuel for boreal wildfires (Amiro et al. 2001, Turetsky et al. 2010). On the opposite, deciduous stands have higher evapotranspiration rates, drier soils, and faster nutrient turnover, all of which are suboptimal conditions for moss growth (Bonan 1991, Turetsky et al. 2010). These plant-soil feedbacks carried out by bryophytes through biomass production support the maintenance of alternative stable states of coniferous and deciduous forests in boreal Alaska.

Forest composition also affected biological N_2-fixation by feather moss-associated cyanobacteria. There has been little done on N_2-fixation associated with mosses in the boreal forest of interior Alaska since the late 1970s (Billington and Alexander 1978) and my results provided a first attempt at quantifying the importance of this process under alternate canopy types. I compared fixation rates in two common feather mosses in the Alaskan boreal forest (*P. schreberi* and *H. splendens*), and how this varied among coniferous and deciduous forest types, over the growing season, and across a nutrient availability gradient. My results suggested that

contribution of feather mosses to vegetation N requirements remained low in birch stands (< 1%), and varied greatly in spruce stands (2-92%). Inter-annual variation, likely associated to precipitation patterns, was the main driver of biological N_2-fixation rates, but my results also suggested that moss species, canopy type, and other environmental factors influence N_2-fixation rates in Alaskan boreal forests. This process may be a significant source of N in coniferous boreal forests, while its importance is likely negligible in deciduous stands. My results enhance the knowledge of the processes that drive N_2-fixation in boreal forests, which is important for predicting ecosystem consequences of changing forest composition. Future research should address the respective impacts of canopy-associated environmental conditions and leaf litter inputs on N_2-fixation rates, as well as including a wider variety of bryophyte species or successional stages.

5.4 Biotic mechanisms and plant-plant interactions control bryophyte communities

I used field-based experiments to assess if differences in moss (*H. splendens*) growth and abundance between deciduous and coniferous forests is attributable to the direct impacts of leaf litter or to other stand-associated environmental conditions. While environmental conditions associated with stand types had an impact on moss growth, my results suggested that deciduous broadleaf leaf litter had the largest detrimental impact on moss health, architecture, and sporophyte production. This study provided experimental support to the long-standing hypothesis that leaf litter is the main mechanism limiting moss abundance in deciduous broadleaf stands (e.g., Van Cleve et al. 1983b). Experimental and ambient leaf litter inputs reduced moss growth and biomass accumulation by approximately 50%, and significantly reduced moss greenness (health) and sporophyte production. These results contrasted with results from subarctic forests in Scandinavia where leaf litter impacts were found to have a positive impact on N_2-fixation (and potentially moss health) (Gundale et al. 2009, Sorensen and Michelsen 2011). This difference was likely due to the large difference in leaf litter input rates between subarctic Scandinavia (75 g m^{-2} $year^{-1}$, ~40% cover; Sorensen and Michelsen 2011) and boreal Alaska (224.8 g dry leaf litter m^{-2} $year^{-1}$, ~100% cover). This supports the idea that there is a threshold in leaf litter inputs that leads to significant detrimental impacts on feather mosses.

The negative impacts I observed were mostly associated with the physical and mechanical effects of leaves such as shading, barrier to vertical growth, and crushing, rather than chemical impacts from leaf leachates. Moreover, leached leaves had a negative impact on the same order as natural leaf litter, which suggests that birch leachates may not be directly as harmful to feather moss growth as those of aspen (Startsev et al. 2008). Detailed comparisons of the chemical composition of birch and aspen leaves are necessary to test this hypothesis. Mosses under high leaf litter cover also presented a high cover of fungi, thereby amplifying the negative effect of leaf litter. I suspect that increases in nutrient availability from easily decomposable birch litter or changes in moisture and light availability that weakened mosses could have unbalanced competitive interactions between parasitic or saprobic fungi. These results suggest that, unlike what has been previously hypothesized, impacts or leaf litter may also be indirect through altered biotic interactions in the moss canopy. Further studies untangling the shading and weight impacts of leaf litter, leachates applications in more controlled conditions, chemical analyses of birch leachates, and detailed investigation of the species interactions among fungi and mosses are future steps that will help clarify the relative importance of the pathways through which leaf litter reduces moss growth and abundance.

5.5 Moss-associated feedbacks in a changing boreal forest

Historically, plant-soil feedback associated with pure deciduous and coniferous forests have favoured their resilience and self-replacement following large stand-replacing fires (Chapin et al. 2010, Johnstone et al. 2010a, Johnstone et al. 2010b). However, northern latitudes have experienced unprecedented warming over the past century, a trend that is predicted to continue (IPCC 2013, ACIA 2005). Recent increases in fire severity in northwestern Canada and Alaska have led to a switch in canopy dominance from black spruce to broadleaf deciduous stands dominated by aspen (*Populus tremuloides* Michx.) or Alaska paper birch (*Betula neoalaskana* Sarg.) (Johnstone et al. 2010a). Deeper combustion of soil organic layers brought about by more severe fires exposes mineral soils on which small-seeded and wind-dispersed deciduous species are able to establish and have a competitive advantage over black spruce (Johnstone and Chapin 2006, Greene et al. 2007). Taking into account the results from this study, I expect that an increase in deciduousness in the landscape (Mann et al. 2012) is very likely to alter moss post-

fire succession, abundance, growth and role in ecosystem processes. This increase in deciduousness can occur both through an increase in pure deciduous stands and an increase in the presence of stands where black spruce shares canopy dominance with either aspen or birch. A moderate input of deciduous broadleaf litter as found in mixedwood stands could have positive impacts on mosses through increased nutrient availability (Oechel and Van Cleve 1986, Sveinbjörnsson and Oechel 1992, Sorensen and Michelsen 2011). There currently is little information regarding the dynamics of mixedwood stands in interior Alaska (Youngblood 1995, Kurkowski et al. 2008). An increase in deciduousness is also likely to affect bryophyte communities by having detrimental impacts on moss establishment from spores or fragments, and decreasing the importance of late successional species such as feather mosses and Sphagna, while post-fire colonists will be able to remain present in pure deciduous and mixedwood stands until the leaf litter cover reaches about 75%. However, these bryophyte communities dominated by *C. purpureus, L. pyriforme, M. polymorpha* and *Polytrichum* spp. do not have the same ecosystem impacts as do feather mosses or Sphagna in terms of insulation, water retention, productivity, and possibly N_2-fixation (Cornelissen et al. 2007, Gavazov et al. 2010, Elumeeva et al. 2011, Soudzilovskaia et al. 2013). In black spruce stands, the high feather moss net primary production, accumulation of thick organic layers promotes further dominance of black spruce (Fenton et al. 2005, Hollingsworth et al. 2006, Johnstone et al. 2010a). Reduced moss cover in deciduous stands leads to warm and dry soils, high nutrient availability, and shallow organic layers, all of which are conditions that are favourable to the stabilization of this alternate state of forest, and making the return to black spruce stands unlikely (Johnstone et al. 2010a). It is also unlikely that permafrost will recover after fire in a stand that experienced a switch in canopy dominance towards more deciduous trees given that the insulating moss layer will not re-establish (Viereck et al. 2008). Changes to feather moss growth and biomass accumulation, and therefore on their role in C cycling, induced by forest type and leaf litter may have cascading impacts on forest structure and composition.

Although much still remains to do in bryophyte ecology in boreal forests, the results presented in this thesis provide a strong framework and basis of empirical data from which to expand. These results provide strong evidence that the interactions between canopy traits (mostly deciduous broadleaf litter production) and bryophytes traits (such as building of organic layers) are key mechanisms supporting plant-soil feedbacks driving the resilience of coniferous and

deciduous alternate stable states in the boreal forest (Johnstone et al. 2010a). My findings also highlight the importance of considering bryophytes as we are trying to predict the future of boreal ecosystems under a changing climate.

5.6 References

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APPENDIX 1: SUPPORTING INFORMATION FOR CHAPTER 2

Table A1.1: Characteristics of 83 forest stands sampled across boreal forests of interior Alaska, grouped by fire name (for sites located in known burned areas) or as mature stands (unburned since at least 1945).

Fire name	Burn year	Year sampled	Since fire (yr)[a]	Slope (°)	Orientation (°)	Elevation (m)	Forest type[b]	DecIV[c]	Black spruce biomass (g/m^2)[d]	Alaska paper birch biomass (g/m^2)[d]	Trembling aspen biomass (g/m^2)[d]	Large shrubs biomass (g/m^2)[e]	Leaf litter cover (%)	Bryophyte cover (%)	Organic layer depth (cm)
Parks Highway	2006	2014	8	0	Flat	125	PM	2.35	8.5	6.3	0.8	59.4	69.6	72	10.0
	2006	2014	8	0	Flat	141	BN	2.38	4.8	13.2	4.3	21.6	44.8	94.4	10.4
	2006	2014	8	0	Flat	142	BN	2.66	2.7	7.1	0.7	23.5	70.4	49.6	12.0
Boundary	2004	2013	9	3	139	489	BN	2.67	8.2	134.8	50.9	38.8	56.8	74.4	8.9
	2004	2013	9	13	225	256	BN	2.82	50.6	1209.6	263.4	76.7	80.8	73.6	11.4
	2004	2013	9	4	203	240	PM	1.9	28.2	7.7	11.5	15.2	74.4	60	16.1
	2004	2013	9	13	315	260	PT	1.8	1.0	0.0	1.0	17.0	58.4	85.6	18.1
	2004	2013	9	6	135	311	PT	2.74	11.4	84.4	241.4	31.1	67.2	86.4	8.8
Fish Creek	2005	2014	9	0	Flat	296	PM	2.3	21.6	18.9	0.3	115.0	69.6	83.2	8.1
	2005	2014	9	4	270	267	BN	2.44	22.0	108.2	4.3	512.3	51.2	84	8.1
	2005	2014	9	2	203	224	PM	2.2	53.0	12.9	0.3	177.3	63.2	61.6	4.2
Chicken 1	2004	2014	10	4	315	718	PT	2.99	0.6	7.1	13.4	138.8	81.6	72	5.9
	2004	2014	10	0	Flat	651	PM	2.88	3.5	0.0	0.0	202.7	72	68	9.6
	2004	2014	10	4	180	657	PT	2.63	7.5	0.0	17.1	40.7	80	60.8	7.9
Porcupine	2004	2014	10	6	225	927	PT	2.97	0.3	0.0	42.0	106.4	70.4	68.8	6.8
	2004	2014	10	5	45	804	PT	2.83	3.6	1.5	10.7	FALSE	76	44.8	7.7
	2004	2014	10	5	45	708	BN	2.66	35.6	169.9	21.4	151.2	60.8	63.2	7.3
	2004	2014	10	8	135	622	BN	2.66	29.3	142.5	22.4	132.7	60	89.6	7.5
Westfork	2002	2014	12	0	Flat	302	BN	2.26	1.5	4.6	0.3	1.1	74.4	61.6	11.7
	2002	2014	12	0	Flat	319	PM	2.17	618.8	1.2	16.5	328.5	65.6	54.4	9.6
	2002	2014	12	25	135	341	BN	2.37	12.8	86.8	0.1	151.2	64	51.2	12.2
Donnelly Flats	1999	2014	15	0	Flat	459	PT	1.67	18.4	1.5	54.4	21.4	88.8	82.4	3.9
	1999	2014	15	0	Flat	521	PM	1.46	82.6	0.0	3.2	0.6	64	87.2	10.0
	1999	2014	15	0	Flat	457	PT	2.44	31.3	1.0	108.9	69.0	64	80.8	3.6
	1999	2014	15	0	Flat	477	PT	2.23	139.4	123.3	636.7	708.0	70.4	63.2	6.0
Hajdukovich	1994	2013	19	0	Flat	468	PT	2.61	8.0	4.4	26.8	66.7	84.8	60	10.2
	1994	2013	19	0	Flat	456	PT	2.53	84.5	2.2	1307.4	179.2	77.6	45.6	4.2
	1994	2013	19	0	Flat	466	PT	2.37	198.9	91.5	229.9	200.3	75.2	67.2	9.1
	1994	2013	19	0	Flat	421	PT	2.72	155.5	15.8	242.6	602.3	77.6	44.8	7.3
Tok River	1990	2010	20	1	297	500	PT	2.89	28.8	0.0	1659.2	863.4	85.6	8	8.0
	1990	2010	20	0	Flat	504	PM	1.82	768.3	1.7	23.6	54.8	32.8	54.4	12.4
	1990	2010	20	0	Flat	503	PT	2.23	183.0	0.0	219.8	106.9	77.6	24	7.7
Granite Creek	1987	2008	21	1	54	385	PT	2.59	76.6	13.9	2135.8	141.8	59.2	20	3.0
	1987	2008	21	0	45	383	PT	2.72	26.9	0.0	290.1	147.4	30.4	43.2	8.0
	1987	2008	21	1	20	436	PM	1.98	159.2	0.0	91.4	132.9	41.6	26.4	6.0
Slate Creek	1987	2009	22	8	68	550	PM	0.21	276.4	0.0	0.0	0.3	36.8	68	18.8
	1987	2009	22	5	64	572	PT	3	0.0	581.3	2820.6	3.2	88.8	0.8	2.6
	1987	2009	22	5	109	553	BN	1.55	168.6	268.9	0.0	0.0	46.4	68.8	11.5
Rosie Creek	1983	2009	25	6	154	199	PM	2.57	174.2	107.1	0.0	447.1	58.4	60.8	16.9
	1983	2009	25	10	154	212	PM	1.88	898.1	83.3	0.0	869.6	34.4	60	10.0
	1983	2009	25	5	128	210	PM	1.53	595.2	140.9	0.0	93.5	49.6	24	7.8
Minto Flats	1983	2009	26	11	99	417	PT	2.91	12.5	1.0	1756.4	0.0	61.6	43.2	1.4
	1983	2009	26	9	103	428	PM	1.18	56.2	0.7	0.0	1.1	48.8	51.2	6.0
	1983	2009	26	15	122	473	PT	2.4	194.2	16.3	1482.6	0.0	76	23.2	3.6
Bolio	1981	2010	29	1	308	496	PT	2.36	141.3	0.0	986.8	17.0	88.8	34.4	3.2
	1981	2010	29	2	301	507	PT	2.72	137.5	0.0	3824.6	0.0	96.8	18.4	6.4
	1981	2010	29	2	301	496	PM	1.68	405.2	1.4	5.3	26.6	83.2	32.8	9.2

Fire name	Burn year	Year sampled	Since fire (yr)[a]	Slope (°)	Orientation (°)	Elevation (m)	Forest type[b]	DecIV[c]	Black spruce biomass (g/m^2)[4]	Alaska paper birch biomass (g/m^2)[d]	Trembling aspen biomass (g/m^2)[d]	Large shrubs biomass (g/m^2)[e]	Leaf litter cover (%)	Bryophyte cover (%)	Organic layer depth (cm)
Wickersham Dome Exp.	1978	2009	31	9	140	494	BN	2.66	208.2	4508.6	0.0	464.0	72.8	16.8	7.4
	1978	2009	31	9	124	494	PM	1.73	1019.4	554.2	0.0	761.8	64	39.2	5.5
	1978	2009	31	8	130	522	PT	1.97	219.9	0.0	3407.5	378.0	76	25.6	3.8
	1978	2009	31	12	87	515	PT	2.16	347.5	90.1	505.2	508.8	43.2	53.6	4.0
	1978	2009	31	8	108	521	PM	1.2	304.2	1.9	0.0	116.2	44.8	44.8	9.4
	1978	2009	31	10	108	509	PM	1.17	482.1	33.1	1.1	247.5	23.2	53.6	6.6
	1978	2009	31	7	105	537	BN	2.46	190.1	486.8	34.3	282.5	67.2	47.2	5.0
Wickersham Dome	1971	2010	37	1	203	526	PM	0.49	469.2	0.0	0.0	17.6	47.2	42.4	7.2
	1971	2010	37	11	176	483	PT	2.61	454.7	105.5	4110.3	33.9	92	5.6	7.0
	1971	2010	37	11	235	403	BN	1.19	403.6	693.5	0.0	14.5	50.4	56.8	10.6
Big Denver	1969	2008	39	0	234	125	PM	1.39	2187.2	0.0	925.3	75.2	50.4	36.8	6.0
	1969	2008	39	0	192	136	PT	2.04	419.9	0.0	2852.5	71.3	81.6	10.4	5.0
	1969	2008	40	8	160	221	BN	3	0.0	10528.6	0.0	583.7	97.6	3.2	2.8
	1969	2008	40	7	255	269	PM	2.13	2696.0	2030.1	0.0	821.2	84	49.6	12.0
	1969	2008	41	5	206	345	PT	2.59	157.5	39.0	5357.4	309.1	93.6	18.4	4.6
Goldstream	1966	2009	43	1	38	144	PM	1.7	3223.2	1973.2	0.0	573.8	67.2	47.2	8.6
	1966	2009	43	3	276	144	PM	1.87	2938.0	0.0	0.0	1287.2	64.8	71.2	15.0
Chena Dome	1958	2009	50	3	145	229	PM	0.94	2168.0	0.0	0.0	250.7	16.8	87.2	14.0
Healy	1958	2008	50	6	149	642	PM	1.53	3873.9	1483.6	1323.6	43.8	48	84	7.4
Murphy Dome	1958	2009	51	7	45	208	BN	2.99	0.0	11614.5	0.0	0.0	93.6	3.2	4.6
	1958	2009	51	6	53	197	BN	1.99	2844.8	8613.2	0.0	0.0	87.2	41.6	13.8
	1958	2009	51	6	11	200	PM	1.1	959.2	24.4	0.0	3.6	45.6	93.6	23.2
Sawtooth Mountain	1957	2009	52	4	286	502	PM	1.7	1520.5	557.6	1387.6	230.7	74.4	51.2	5.2
	1957	2009	52	4	213	489	PM	1.28	1683.0	106.3	86.2	151.8	52	28.8	4.0
Big Gerstle	1947	2009	62	1	3	493	BN	1.94	1265.7	7055.2	1824.2	1201.7	91.2	20.8	4.0
	1947	2009	62	1	344	506	PT	2.49	17.2	3020.1	9601.5	337.7	84	32.8	2.4
	1947	2009	62	2	15	535	PM	1.58	765.0	195.1	0.0	35.0	37.6	61.6	17.8
Mature deciduous 4	NA	2009	70	4	135	288	BN	2.65	514.7	13456.3	0.0	1522.8	96.8	0	14.6
Mature deciduous 2	NA	2009	74	4	135	433	BN	2.4	0.0	9388.5	0.0	74.2	96	0	7.8
Mature spruce 1	NA	2009	78	9	20	400	PM	1.44	2695.9	297.0	0.0	48.7	39.2	96	27.8
Mature deciduous 1	NA	2009	84	5	90	489	PT	2.78	4.0	0.0	10391.8	0.0	91.2	17.6	4.5
Mature spruce 4	NA	2009	85	5	54	270	PM	0	5127.8	0.0	0.0	0.0	31.2	96	24.0
Mature deciduous 3	NA	2009	87	4	135	265	BN	3	0.0	19949.1	0.0	93.6	95.2	13.6	9.0
Mature deciduous 5	NA	2009	120	6	11	519	PT	2.67	12.1	0.0	12712.6	111.9	88.8	44.8	4.4
Mature spruce 3	NA	2009	138	0	0	485	PM	0	4906.9	0.0	0.0	0.0	12.8	98.4	28.6
Mature spruce 2	NA	2009	163	0	0	480	PM	1.62	6585.0	0.0	0.0	52.9	48	86.4	25.4

NA = No data. BN = Alaska paper birch, PM = black spruce, PT = trembling aspen. Flat = Stands were on flat ground so do not have slope or aspect.

[a] Years since fire (age) based on sampling year minus year burned from historic fire records, or tree rings in the case of some of the oldest stands.

[b] Forest type based on the dominant contribution to total standing biomass. See Alexander et al. (2012) for calculation.

[c] Deciduous Importance Value (IV). See Alexander et al. (2012) for calculation.
[d] Estimated from density and basal area using allometric equations presented in Alexander et al. (2012).
[e] Estimated from density and basal area of large shrubs (*Alnus* spp., *Salix* spp. and *Betula* spp.) using allometric equations in Berner et al. (2015).

Table A1.2: Results from the two-way ANOVA on total bryophyte cover compared between the three forest types (black spruce, Alaskan paper birch, and trembling aspen) and the three post-fire successional periods (early, mid-, and late succession). This ANOVA used a type III sums of squares in R to address the unbalanced data.

	Sum of squares	**Degrees of freedom**	**F value**	***p* value**
Forest type	137	2	0.236	0.791
Successional stage	12414	2	21.345	<0.0001
Interaction	10836	4	9.316	<0.0001
Residuals	21519	74		

Table A1.3: Results from the two-way unbalanced ANOVAs by permutation (4999) conducted on the three major bryophyte functional groups compared between the three forest types (black spruce, Alaskan paper birch, and trembling aspen) and the three post-fire successional periods (early, mid-, and late succession). This ANOVA used a type III sums of squares in R to address the unbalanced data.

a) Colonizers

	Mean squares	**Degrees of freedom**	**F statistic**	***p* value**
Forest type	1.494	2	0.251	0.7776
Successional stage	545.229	2	91.435	0.0002
Interaction	2.834	4	0.916	0.9154
Residuals	220.632	74		

b) Feather mosses

	Mean squares	**Degrees of freedom**	**F statistic**	***p* value**
Forest type	53.545	2	14.272	<0.0001
Successional stage	86.584	2	23.079	<0.0001
Interaction	75.869	4	10.111	<0.0001
Residuals	138.810	74		

c) Acrocarpous mosses

	Mean squares	**Degrees of freedom**	**F statistic**	***p* value**
Forest type	20.517	2	10.460	0.0014
Successional stage	0.159	2	0.081	0.9248
Interaction	0.631	4	0.161	0.9488
Residuals	72.573	74		

Table A1.4: Results from the two-way unbalanced ANOVA on taxa composition data compared between the three forest types (black spruce, Alaskan paper birch, and trembling aspen) and the three post-fire successional periods (early, mid-, and late succession).

a) Results from the 2 way unbalanced ANOVA with permutations (4999) on taxa composition data. Similar results were obtained by using a Hellinger transformation on species data, or using a permutation ANOVA with Bray-Curtis distance not accounting for unbalanced data.

	Degrees of freedom	**Variance**	**F statistic**	***p* value**
Forest type	2	93.71	3.729	0.0006
Successional stage	2	438.75	17.461	0.0002
Interaction	4	110.28	2.194	0.0030
Residuals	74	929.72		

b) ANOVA by permutations (4999) comparing bryophyte taxa composition among forest types within the early succession stands.

	Degrees of freedom	**Sum of squares**	**F statistic**	***p* value**
Forest type	2	0.443	1.888	0.0572
Residuals	26	3.049		
Total	28	3.491		

c) ANOVA by permutations (4999) comparing bryophyte taxa composition among forest types within the mid-succession stands, followed by pairwise comparisons using the Bonferonni correction for multiple comparisons.

	Degrees of freedom	**Sum of squares**	**F statistic**	***p* value**
Forest type	2	1.394	2.848	0.0004
Residuals	39	9.542		
Total	41	10.935		

Post-hoc pairwise comparisons	**F statistic**	**Adjusted *p* value**
Spruce vs. Aspen	4.171	0.009*
Spruce vs. Birch	1.506	0.471
Aspen vs. Birch	2.306	0.141

* indicates significance at α=0.05

d) ANOVA by permutations (4999) comparing bryophyte taxa composition among forest types within the late succession stands, followed by pairwise comparisons using the Bonferonni correction for multiple comparisons.

	Degrees of freedom	**Sum of squares**	**F statistic**	***p* value**
Forest type	2	4.429	4.298	0.0008
Residuals	9	1.497		
Total	11	2.916		

Post-hoc pairwise comparisons	**F statistic**	**Adjusted *p* value**
Spruce vs. Aspen	3.849	0.153
Spruce vs. Birch	5.785	0.030
Aspen vs. Birch	2.601	0.585

APPENDIX 2: COMPARISON OF METRICS OF MOSS SPECIES COMPOSITION, HEALTH AND GROWTH OF *HYLOCOMIUM SPLENDENS*

A2.1 Methods

A2.1.1 Species composition

Moss species composition in the transplant experiment was monitored using point-intercept sampling (Goodall 1952) with a grid of 100 points, of which ~76 points intersected the sampling unit (SU; transplanted moss core or procedural control). The grid was positioned in the same place over the transplant for assessments in September 2012 and July 2015. I truncated observations to the eight most common and large species of mosses and lichens (*Hylocomium splendens* (Hewd.) Schimp., *Pleurozium schreberi* (Brid.) Mitt., *Polytrichum commune* Hewd., *Dicranum* spp., *Ptilium crista-castrensis* (Hewd.) De Not., *Sphagnum* spp., *Peltigera aphthosa* (L.) Willd., and *Peltigera malacea* (Ach.) Funck. cf.) to control for differences in detection rate of rare and inconspicuous species between the two sampling years. As I recorded species composition in 2012 and 2015, I visually attributed a colour to the dominant *H. splendens* as being either green or brown. Observations were made by the same person in both years to limit observer bias. Only the green *H. splendens* was included in the species composition data file, as I assumed that the brown ones were either dead or dying.

A2.1.2 Health (greenness)

Moss greenness is an indicator of moss health, water content, and likely related to chlorophyll content (Olthof and Latifovic 2007, Malenovsky et al. 2013). I used three different methods to assess greenness of the SUs.

A2.1.2.1 Visual estimates

I compared the cover of green and brown *H. splendens* from the species composition records in 2012 and 2015. I also recorded the colours of all the segments from the tagged individuals collected in August 2015 using the colour chart from Dignard et al. (2013).

A2.1.2.2 Digital photos

Pictures of all the SUs without leaf litter were taken using digital cameras in September 2012 and August 2015. I calculated a numerical value of greenness for each colour using the Dark Green Colour Index (DGCI; Karcher and Richardson 2003) on the average SU colour measured in Adobe Photoshop CS5 (Adobe Systems, Mountain View, CA). This index was obtained by extracting the average hue, saturation and brightness for each SU. As mosses are typically not as deep green as vascular plants, I used a modified DGCI index by lowering the

optimal hue of green (more yellow). A value of 1 corresponded to an optimal moss green colour, and a value of 0 was not green. Not all pictures were taken with the same camera, or under a similar lighting. However, I assumed that discrepancies in baseline light levels of camera were accounted for by the inclusion of the random effects of block, forest type, and plot, since sampling was nested in space and time and light did not vary noticeably during a sampling event.

A2.1.2.3 NDVI

In September 2013 and July 2015, I took three pictures of each SU without leaf litter using a Tetracam camera (Tetracam inc, USA), which allowed measurements of the Normalized Difference Vegetation Index (NDVI). All the pictures were analyzed using PixelWrench2 (Tetracam inc, USA) to obtain the average NDVI value. High NDVI (near 1) indicated a greener colour in the selected area of the picture. There was one missing value for 2015, and I filled it in with the average NDVI value in order to keep the balance of the experimental design.

A2.1.3 Growth

Three different methods were used to measure moss growth that varied in precision and time requirements.

A2.1.3.1 Sampling unit height from the forest floor

I assessed the height of the SU relative to the surrounding ground using angle brackets and rulers. Three measurements points were taken around each SU in June 2013, 2014 and 2015.

A2.1.3.2 Cranked wire

A cranked wire transect was installed in each SU in June 2013, based on the method developed by Clymo (1970) to measure *Sphagnum* spp. The cranked wire transect was built using two ~15 cm long galvanized steel wires installed parallel to and at the moss surface. Ten measures of the moss height relative to the wire were taken in June and October 2013, and were repeated in 2014 and 2015.

A2.1.3.3 Tagged individuals

I marked five randomly selected *H. splendens* shoots in SU of the moss transplant experiment (n = 120 * 5 = 600) in June 2013, and an additional five random shoots per SU in September 2013 (total n = 1200). Ten individuals of *H. splendens* and five of *P. schreberi* were tagged in replicate SU of the leachates experiment in September 2014. I used PVC rings (HAMA plastic beads, Malte Haaning Plastics Co., Denmark; outer diameter 2.5 mm and inner diameter 1

mm) with a slit that allowed the rings to be placed as markers on moss shoots using tweezers. Locations of individuals were recorded using a grid system, which allowed me to re-measure the same individual multiple times. I measured width and length of each step from the marked individuals in June and September 2013, June and September 2014, and June and late August 2015 for the transplant experiment, and September 2014 and 2015 for the leachates experiment. An additional 5 shoots of *Pleurozium schreberi* were tagged in each SU of the leachates experiment in June 2015 and re-measured in August 2015. All shoot were destructively harvested in August 2015 and the final sample weights and dimensions were measured in the lab. Moss biomass estimates for 2013 and 2014 were obtained using an allometric equation developed based on moss area using independent moss samples (Appendix 3).

A few variables were calculated using the measurements on the tagged individuals. I estimated the total area (sum of the length * width / 2 of moss segments), moss biomass (dry weight), and number of moss segments for each individual moss shoot. All variables were calculated both for the total moss shoot and for the dominant chain of segments only. This distinction was included to account for a possible lower probability of detection of side branches in the field, compared to the final lab measurements. All values were averaged per SU instead of keeping the raw data on the 4-10 moss shoots measured per SU, in order to limit the issues related to missing data caused by lost, broken, or found again moss individuals.

A2.1.4 Data analysis

All analyses were completed using R (R Core Team 2016).

A2.1.4.1 Species composition

Changes in species composition in the sampling units were assessed using multivariate ANOVAs by permutations. I created a new vector with eight levels representing the interaction between forest type and leaf litter treatment (Forest-Treatment). For the within-subject effect (year), I used a permutation multivariate ANOVA (permanova) using the *adonis* function in the package *vegan* (Oksanen et al. 2016) and the Bray Curtis distance. This function allowed me to test for the year and Forest-Treatment*year effects, along with a random sampling unit identifier to account for repeated measures (nested within Forest-Treatment). I also used a permanova using the *nested.npmanova* function from the *BiodiversityR* package (Kindt and Coe 2005) in order to accurately estimate the main effect of Forest-Treatment. Data were graphically

represented using a non-metric dimensional scaling (NMDS) using the package *vegan* (Oksanen et al. 2016). Environmental variables were correlated to the main NMDS axes to help explain the observed patterns.

A2.1.4.2 Health (greenness)

The colour obtained through the collected samples were not subjected to statistical analysis, but simply graphed according to moss growth and leaf litter cover. I obtained the hue, saturation, and brightness from the colours on the dominant segment of all samples using the cohort marked with a yellow bead (2013 growth, Figure 3.2), and then calculated the average moss colour per transplant.

I analyzed the three moss greenness indicators in 2015 using linear mixed effects models (library *lmerTest*; Kuznetsova et al. 2015) with nested random effects of plot within forest type within blocks, followed by Tukey Honest Significant Difference (HSD) post-hoc tests when applicable. The fixed effects were forest type, treatment or leaf litter cover and the quadratic term of leaf litter cover, and greenness at the start of the experiment (2012 or 2013). This last effect accounted for the influence of initial moss colour (and therefore health and growth) on 2015 colour. Adding this factor improved all the models significantly based on Akaike's Information Criterion (AIC) (Crawley 2007). Six models were therefore created. In the case of the models including leaf litter, the fixed effects were scaled prior to data analysis to avoid having variables on very different scales and to facilitate effect size comparisons (Bolker 2016). The NDVI baseline data were taken in 2013, i.e. a year after the first leaf litter addition, I therefore interpreted the results with caution.

A2.1.4.3 Growth

All data from the SU height and cranked wire measurements were centered using the measurements from June 2013 as the baseline. For the six variables measured on the marked individuals, I used the difference between the 2015 and 2013 data as the response variable. Linear mixed effect models (library *lmerTest*; Kuznetsova et al. 2015) were used to compare the change in height between 2015 and 2013 according to forest type and either treatment or leaf litter, as well as their interaction. The nesting of plot within forest type, within block was used to account for the experimental design.

A2.1.4.4 Comparison of different moss measuring methods

I calculated Pearson correlations among all the variables collected, i.e. moss greenness variables in 2015, and the difference between the 2015 and 2013 measurements of moss growth. These correlations indicated how similar all the measurements were, and how well they could be related to estimates of moss biomass growth at the individual moss shoot level.

A2.2 Results

A2.2.1 Species composition

Species composition changed between September 2012 and August 2015, and interacted with forest type and treatment ($p < 0.0001$; Table A2.1). The most obvious difference was between species composition of the controls in birch (more *H. splendens* monospecific) and spruce stands (more mixed with other species such as *Polytrichum* spp. and *Peltigera* spp. lichens) that remained in both years. The first axis of the ordination was mostly associated with the forest type of origin of the sampling unit with procedural controls on the right side of the NMDS plot, while the second axis was positively associated with leaf litter cover (Table A2.1; Figure A2.1). Temporal changes in species composition according to forest type and leaf litter treatment were not as obvious as this baseline difference. Over time, the species composition of the transplants without leaf litter in spruce and birch stands changed slightly to become more similar to that of the spruce procedural controls with more *H. splendens*, *P. schreberi* and *Sphagnum* spp. Transplants with ambient and experimental leaf litter inputs remained more similar to their initial species composition, with more *Peltigera aphtosa* and *Polytrichum commune* (Figure A2.1). These sampling units were characterized by a lower cover of green *H. splendens*.

Table A2.1: Results from the two complementary permanovas conducted to test for the impacts of forest type and leaf litter treatment (as one variable with eight levels, i.e. Forest type – Treatment).

a) Output from the *adonis* function to test for the year and interaction effects.

	Degrees of freedom	**Sums of Squares**	**F value**	***p* value**
Forest type - Treatment	7	2.9385	24.9871	.[a]
Year	1	0.4917	29.2677	<0.0001
Sampling unit	112	8.226	4.3717	<0.0001
(Forest type – Treatment)*Year	7	0.7457	6.341	<0.0001
Residuals	112	1.8816	0.13173	
Total	239	14.2835		

b) Output from the *nested.npmanova* function to test for the main effect.

	Degrees of freedom	**Sums of Squares**	**F value**	***p* value**
Forest type – Treatment	7	71220	6.8872	<0.0001
Sampling unit	112	165454	2.7811	.[a]
Residuals	120	63742	531.1852	

[a] The period indicates that no *p*-value could be reliably estimated for this factor.

Table A2.2: Axis loadings of environmental and stand covariates on the two NMDS axes.

	NMDS1	NMDS2	r^2	*p* value
Forest type of origin[a]	0.9952	-0.0981	0.2006	0.001
Leaf litter cover	-0.2781	0.9606	0.0912	0.001
Forest type[a]	-0.5128	-0.8585	0.0906	0.001
Block[b]	0.4544	0.8908	0.0226	0.063
Fungus cover (%)	-0.3890	0.9213	0.1203	0.001
Canopy cover	0.9029	0.4230	0.0470	0.006
Moisture (%)	-0.8585	-0.5128	0.0301	0.020

[a] Paper birch=1, Black spruce =2
[b] Block A=1, Block B=2, Block C=3

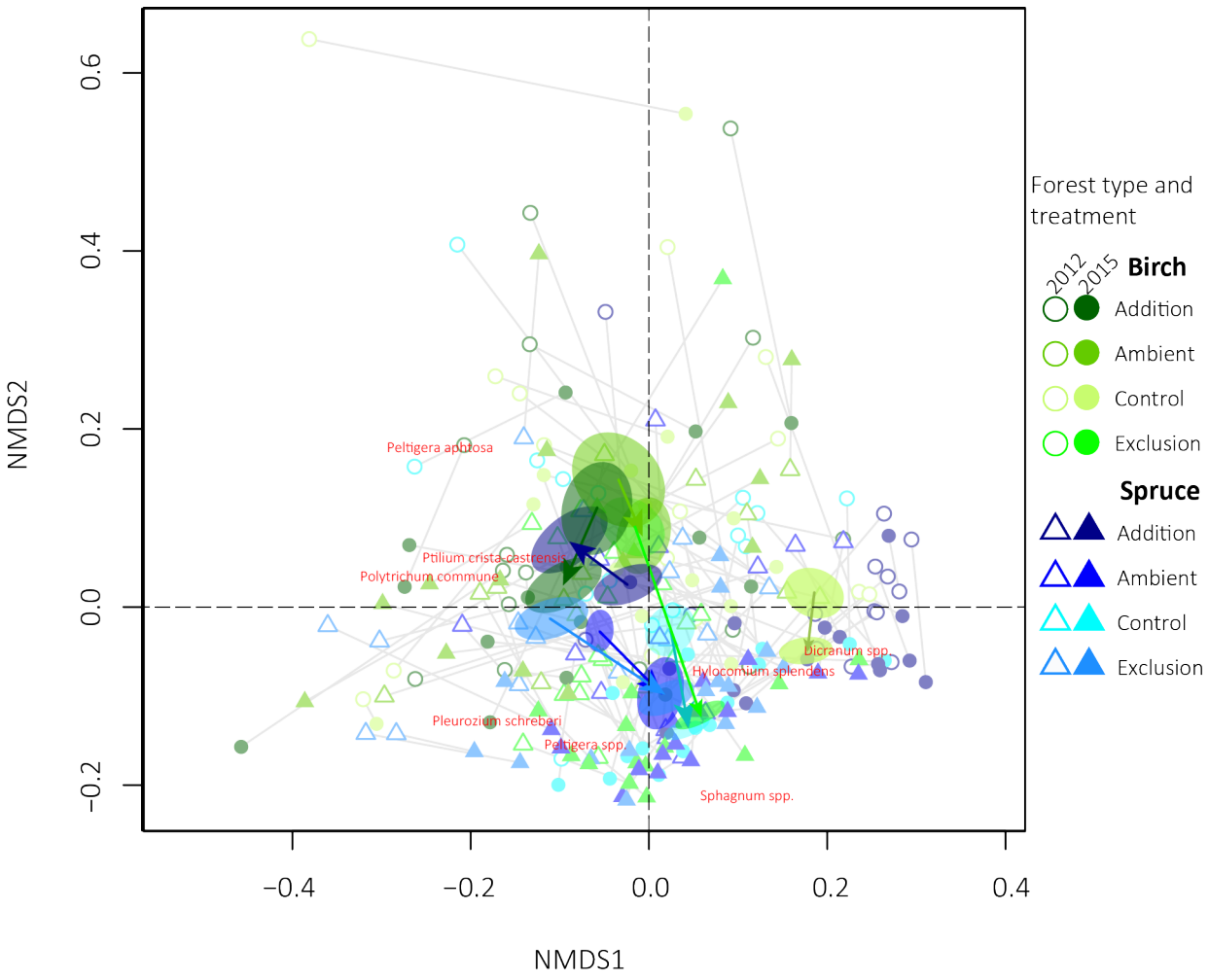

Figure A2.1: Distribution of the sampling units (transplants and procedural controls) in a two-dimensional NMDS ordination (stress of 0.13, 100 iterations, Bray-Curtis distance) based on the community composition of dominant mosses and lichens in 2012 and 2015 (120 transplants*2 years, 8 taxa). The two axes capture 85.3% of the variation in the original ranked matrix, with axes 1 and 2 capturing 41.4% and 43.9% of the variation, respectively. Individual points represent sampling units grouped by forest type: spruce (blue, triangles) and birch (green, circles). The shades represent the leaf litter treatments. Ellipses are drawn for each year*forest type*treatment combination and encompass the average and standard error. Species vectors are only represented by species names in red font. Distance of species from the center was doubled in order to improve readability. Arrows show the change in the centroid of the forest type*treatment group between 2012 (origin) and 2015 (arrow head). Light grey lines link individual sampling units between the two years.

A3.2.2 Moss greenness

Overall, the colour of the yellow segment (~initiated in 2014) on the harvested mosses in August 2015 does not seem to visually indicate a strong relationship between moss colour, leaf litter, and moss growth (Figure A2.2). Mosses seemed yellower in spruce than in birch stands, and in both forest types mosses tended to become greener and darker as leaf litter cover increased. Moss growth did not seem to be related to moss colour (Figure A2.2).

I found that for both models tested, there was a positive association between the percent cover of green *H. splendens* in the SUs in 2012 and in 2015 (Table A2.3a). I found no linear or quadratic relationship between leaf litter cover and moss greenness, but there was a significant treatment effect, with the leaf litter addition treatment being significantly less green than the other ones ($p < 0.0001$; Table A2.3a). Contrary to my personal field observations, I found no effect of forest type on moss greenness measured by this metric (Table A2.3a).

Greenness of the SUs estimated using the DGCI followed a quadratic relationship with leaf litter cover, with mosses being greener at intermediate (up to about 25-30%) leaf litter cover ($p = 0.0436$; Table A2.3b), suggesting an increased chlorophyll content as they try to photosynthesize under reduced light. When looking at leaf litter treatments, SUs were greener in the procedural controls and leaf litter exclusion treatments than in the ambient, and leaf litter addition treatments (Table A2.3b). Mosses were found to be greener in spruce than in birch stands, but only in the model with leaf litter cover ($p = 0.0064$; Table A2.3b), while previous moss greenness was only important in the model that included leaf litter treatment as a categorical variable ($p = 0.0041$; Table A2.3b).

Results from the model focusing on NDVI values and the leaf litter cover variable yielded contradictory results to those of the DGCI models, with the quadratic term of leaf litter being significant, but showing greener values at low and high leaf litter covers ($p = 0.0059$; Table A2.3c). There was an interaction between leaf litter cover and forest type, with a negative impact of leaf litter on NDVI (leading to mosses that are less green) in spruce stands, but no relationship in birch stands ($p = 0.0059$; Table A2.3c). Mosses in the addition treatment were the less green, while the ones in the ambient treatment were the greenest ($p = 0.0051$; Table A2.3c). I did not find a relationship between initial and final NDVI values (Table A2.3c). Overall, I

found that this variable yielded results that were difficult to interpret, and that contradicted both other variables measured as well as my field observations.

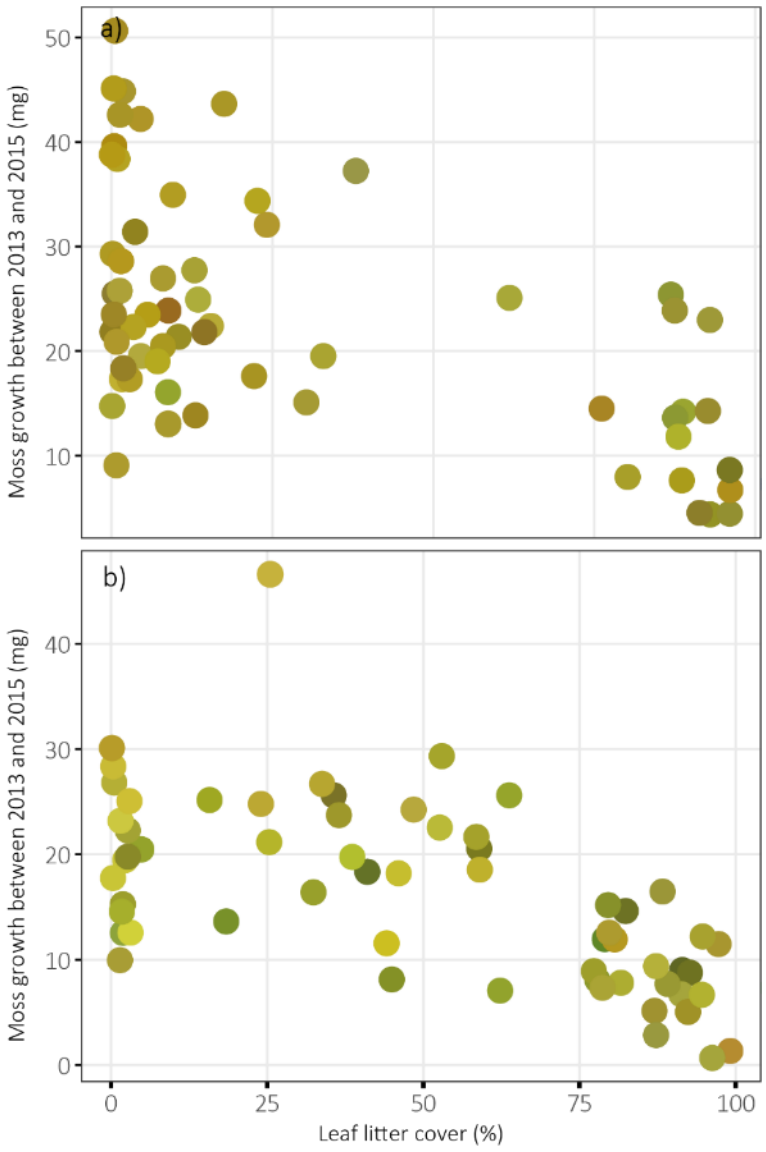

Figure A2.2: Average colour of the moss segment marked by the yellow bead (~ initiated in 2014) on the dominant chain of segments. Each point represents a transplant, and the colour of each point was calculated by taking the average hue, saturation and brightness of the 10 moss shoots. The points are ordered according to leaf litter cover (%) and total moss growth (mg) between 2013 and 2015. (a) Black spruce stands. (b) Alaska paper birch stands.

Table A2.3: Summary of results of statistical analyses from the best models selected to test for effects of forest type, canopy cover, initial colour (2012 or 2013), and either leaf litter cover or treatment on three variables of moss greenness: a) Percent cover of green *Hylocomium splendens* from visual estimates, b) DCGI, and c) NDVI. Test results are summarized by the F statistic, associated probability (*p*) value and degrees of freedom obtained with Satterthwaite estimation for degrees of freedom. For continuous variables, the direction of significant effects is indicated by + or – signs. Significant categorical variables were followed by a Tukey HSD test. Significant effects are shown in bold type ($\alpha = 0.05$).

Leaf litter cover or Treatment used?	Fixed effects	Effect direction	DFn, DFd	F statistic	*p* value
a) Visual estimates					
Leaf litter cover	Leaf litter cover (%)		1, 97.1	0.382	0.5378
	Leaf litter cover (%)^2		1, 97.9	2.951	0.0900
	Forest type		1, 32.3	< 0.001	0.9868
	Green cover 2012 (%)	+	**1, 108.4**	**12.538**	**0.0006**
	Leaf litter cover (%) * Forest type		1, 89.3	1.235	0.2694
Treatment	**Treatment**	**ADD < AMB, EXC, CON[c]**	**3, 88.2**	**15.272**	**< 0.0001**
	Forest type		1, 28.0	0.535	0.4705
	Green cover 2012 (%)	+	**1, 110.5**	**10.371**	**0.0017**
b) DGCI[a]					
Leaf litter cover	Leaf litter cover (%)		1, 113.5	1.810	0.1812
	Leaf litter cover (%)^2	**Higher at intermediate values**	**1, 113.8**	**4.163**	**0.0436**
	Forest type	**PM > BN[d]**	**2, 113.8**	**7.721**	**0.0064**
	DGCI 2012		1, 93.3	2.032	0.1574
	Leaf litter cover (%) * Forest type		1, 112.0	2.406	0.1237
Treatment	**Treatment**	**CON, EXC > ADD, AMB[c]**	**3, 112.9**	**13.733**	**> 0.0001**
	DGCI 2012	+	**1, 13.6**	**11.892**	**0.0041**
c) NDVI[b]					
Leaf litter cover	Leaf litter cover (%)	+	1, 100.1	1.341	0.2497
	Leaf litter cover (%)^2	**Lower at intermediate values**	**1, 101.1**	**7.956**	**0.0059**
	Forest type	**PM > BN[d]**	**1, 2.1**	**21.796**	**0.0386**
	NDVI 2013		1, 113.1	0.162	0.6878
	Leaf litter cover (%) * Forest type	**+ for PM, ~0 for BN[d]**	**1, 90.9**	**7.956**	**0.0059**

Leaf litter cover or Treatment used?	Fixed effects	Effect direction	DFn, DFd	F statistic	*p* value
Treatment	**Treatment**	**ADD ≤ CON, EXC ≤ AMB[c]**	**3, 82.6**	**4.583**	**0.0051**
	Forest type	**PM > BN[d]**	**1, 2.0**	**21.619**	**0.0433**
	NDVI 2013		1, 110.3	0.007	0.9361
	Treatment *Forest type		3, 82.1	1.822	0.1496

[a] Dark Green Colour Index (Karcher and Richardson 2003)
[b] Normalized Difference Vegetation Index
[c] Codes for the leaf litter treatments: addition (ADD), ambient (AMB), exclusion (EXC), procedural control (CON)
[d] PM: *Picea mariana*, i.e. black spruce stands; BN: *Betula neoalaskana*, i.e. Alaska paper birch stands

A3.2.3 Growth

The major difference in height of the top of the SU from the surrounding forest floor occurred in the leaf litter addition in spruce stands, with a decrease in height of 5.2 cm ($p < 0.0001$; Table A2.4a; Figure A2.3). Most other SUs showed either little change in their height over time, or a smaller decrease on the order or 1.5 to 2 cm. The results likely highlight the fact that transplants in birch stands, even with high leaf litter inputs, were resting on the mineral soil and could not therefore sink down or collapse too much. This was not the case for the addition treatment in spruce stands where leaf litter inputs on a transplant resting on a decomposing moss matrix promoted a deeper sinking (Table A2.4a).

Through the cranked wire method, I found that mosses grew the best in spruce stands, and in the treatments with low leaf litter inputs like the procedural controls, and exclusion and ambient treatments (Table A2.4b; Figure A2.3). However, I found that the exclusion treatment in birch stands experience a decrease in growth similar to that of the addition and ambient treatments in that stand. Leaf litter had a stronger negative impact on moss growth in spruce than in birch stands ($p < 0.0001$; Table A2.4b), but the change in growth over time in the addition treatment in spruce stands is very minimal (~0.1 cm; Figure A2.3).

Table A2.4: Summary of results of statistical analyses from the models looking at the effects of forest type, leaf litter or treatment, and their interaction on a) distance of the moss canopy from the forest floor (cm), and b) distance from the moss canopy from the cranked wire (cm). Test results are summarized by the F statistic, associated probability (*p*) value and degrees of freedom obtained with Satterthwaite estimation for degrees of freedom. For continuous variables, the direction of significant effects is indicated by + or – signs. Significant categorical variables were followed by a Tukey HSD test. Significant effects are shown in bold type ($\alpha = 0.05$).

Leaf litter cover or Treatment used?	Fixed effects	Effect direction	DFn, DFd	F statistic	*p* value
a) Height of the SU from the forest floor (cm)					
Leaf litter cover	**Leaf litter cover**	**-**	**1, 91.2**	**51.388**	**< 0.0001**
	Forest type		1, 4.7	3.489	0.1241
	Leaf litter cover * Forest type		1, 91.2	3.040	0.0846
Treatment	**Treatment**		**3, 84**	**15.229**	**< 0.0001**
	Forest type		1, 2	5.220	0.1497
	Treatment * Forest type	**See** Figure A3.2	**3, 84**	**9.521**	**< 0.0001**
b) Moss height from the cranked wire (cm)					
Leaf litter cover	**Leaf litter cover**	**-**	**1, 111.3**	**8.430**	**0.0045**
	Forest type	**PM > BN**[a]	**1, 8.7**	**18.709**	**0.0021**
	Leaf litter cover * Forest type	**More negative slope in PM**	**1, 111.3**	**5.555**	**0.0202**
Treatment	**Treatment**	**ADD ≤ AMB EXC ≤ CON**[b]	**3, 107.1**	**3.415**	**0.0201**
	Forest type	**PM > BN**[a]	**1, 2.0**	**21.256**	**0.0433**
	Treatment * Forest type		3, 107.1	1.468	0.2275

[a] PM: *Picea mariana*, i.e. black spruce stands; BN: *Betula neoalaskana*, i.e. Alaska paper birch stands

[b] Codes for the leaf litter treatments: addition (ADD), ambient (AMB), exclusion (EXC), procedural control (CON)

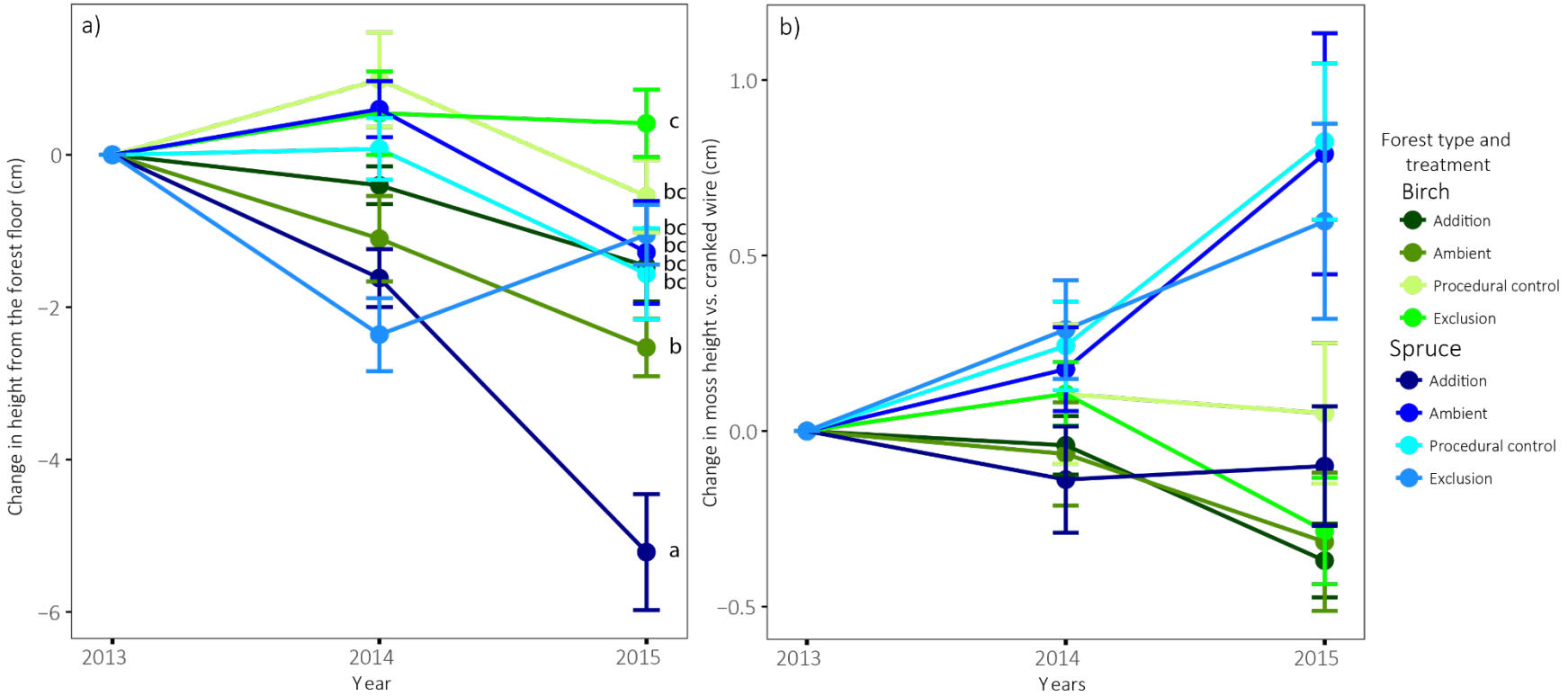

Figure A2.3: Changes in moss growth as measured over three years by a) distance of the moss canopy from the forest floor (cm), and b) distance of the moss canopy from the cranked wire (cm). All values have been centered at 0 based on the data from June 2013 (first measurements). Points represent averages ± standard error and colours indicate the forest type and leaf litter treatment. Different letters next to the 2015 points indicate a significant difference based on a Tukey HSD posthoc test (α=0.05).

Overall, the trends found were very similar among all six variables reprensenting moss growth considered. This was particularly the case with variables calculated on the total moss growth and moss growth only on the dominant chain of segments (Table A2.5; Figure A2.3). Change in moss area and biomass between 2013 and 2013 also lead to very similar results. For those two variables, I found that there was a strong negative impact of leaf litter cover on moss growth (Table A2.5a-b). This impact of leaf litter was also reflected in the significant interaction between forest type and treatment, with the treatments with ambient and experimental leaf litter inputs showing a significantly lower growth (Figure A2.3a-d; Table A2.5a-b). Mosses in spruce stands grew a little more than the ones in birch stands, but this difference was generally not significant (Figure A2.3a-d). Using the number of segments per moss shoot lead to a loss of some of the nuances previously found with the other variables, as well as some more inconsistent results (Figure A2.3e-f; Table A2.5c). The main pattern that still emerged was that mosses under leaf litter inputs produced about one segment less than the mosses not subjected to leaf litter inputs.

Table A2.5: Summary of results of statistical analyses from the models looking at the effects of forest type, leaf litter or treatment, and their interaction on the difference between 2015 and 2013 of moss area (cm^2), moss biomass (mg), and number of segments, both for the total moss shoot and the dominant chain of segments per shoot. Test results are summarized by the F statistic, associated probability (*p*) value and degrees of freedom obtained with Satterthwaite estimation for degrees of freedom. The impact of leaf litter was always negative. Significant categorical variables were followed by a Tukey HSD test based on growth between 2013 and 2015, the results can be found in Figure 3. Significant effects are shown by an asterisk ($\alpha = 0.05$).

		Fixed effects	DFn, DFd	F statistic	*p* value	
Moss area (cm^2)	**a)**	**Total moss area**				
	Leaf litter cover	Leaf litter cover	1, 91.6	80.115	< 0.0001	*
		Forest type	1, 3.6	4.063	0.1212	
		Leaf litter cover * Forest type	1, 91.6	0.240	0.6257	
	Treatment	Treatment	3, 84	29.488	< 0.0001	*
		Forest type	1, 2	9.895	0.0879	
		Treatment * Forest type	3, 84	4.397	0.0064	*
	b)	**Dominant chain of segments moss area**				
	Leaf litter cover	Leaf litter cover	1, 91.3	85.550	< 0.0001	*
		Forest type	1, 4.2	7.390	0.0505	
		Leaf litter cover * Forest type	1, 91.3	0.425	0.5161	
	Treatment	Treatment	3, 84	34.249	< 0.0001	*
		Forest type	1, 2	16.265	0.0563	
		Treatment * Forest type	3, 84	6.239	0.0007	*
Moss biomass (mg)	**c)**	**Total moss biomass (mg)**				
	Leaf litter cover	Leaf litter cover	1, 91.9	73.150	< 0.0001	*
		Forest type	1, 3.8	2.537	0.1870	
		Leaf litter cover * Forest type	1, 91.9	0.302	0.5838	
	Treatment	Treatment	3, 84	23.156	< 0.0001	*
		Forest type	1, 2	7.523	0.1112	
		Treatment * Forest type	3, 84	4.927	0.0034	*
	d)	**Dominant chain of segments moss biomass (mg)**				
	Leaf litter cover	Leaf litter cover	1, 91.6	76.572	<0.0001	*
		Forest type	1, 4.3	5.373	0.0761	
		Leaf litter cover * Forest type	1, 91.7	0.720	0.3983	
	Treatment	Treatment	3, 84	24.936	< 0.0001	*
		Forest type	1, 2	12.658	0.0707	
		Treatment * Forest type	3, 84	5.790	0.0012	*
Number of segments (count)	**e)**	**Total number of moss segments**				
	Leaf litter cover	Leaf litter cover	1, 114.0	1.123	0.2915	
		Forest type	1, 114.0	29.227	< 0.0001	*
		Leaf litter cover * Forest type	1, 114.0	0.915	0.3408	
	Treatment	Treatment	3, 110	11.007	< 0.0001	*
		Forest type	1, 1120	0.915	0.3409	
		Treatment * Forest type	3, 110	2.207	0.0913	
	f)	**Dominant chain number of moss segments**				
	Leaf litter cover	Leaf litter cover	1, 116	53.018	< 0.0001	*
		Forest type	1, 116	0.066	0.7971	

	Fixed effects	DFn, DFd	F statistic	*p* value	
Treatment	Leaf litter cover * Forest type	1, 116	2.185	0.1421	
	Treatment	3, 112	19.495	< 0.0001	*
	Forest type	1, 112	11.328	0.0010	*
	Treatment * Forest type	3, 112	3.979	0.0098	*

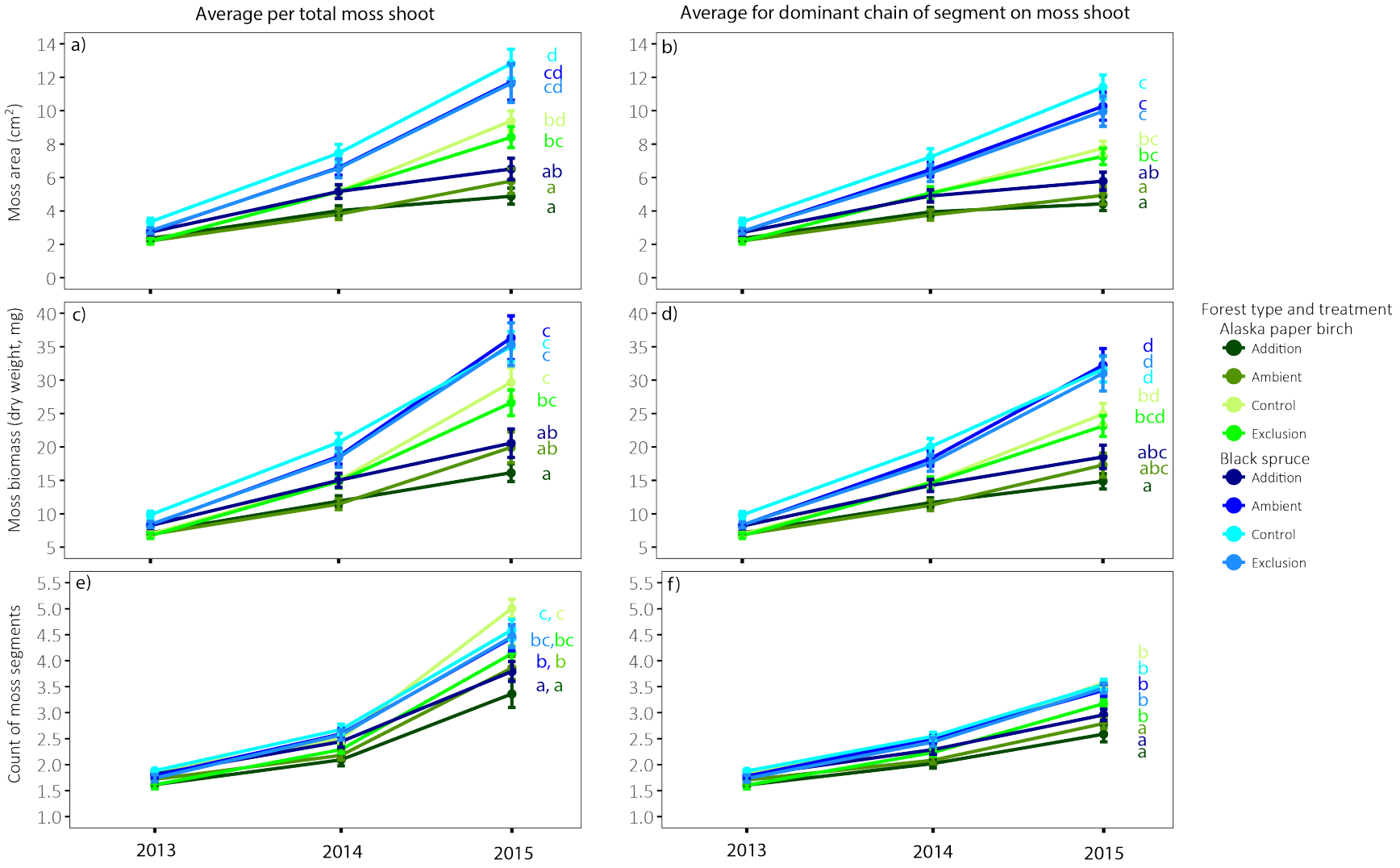

Figure A2.4: Average moss area (cm^2), moss biomass (mg), and number of segments, both for the total moss shoot and the dominant chain of segments per shoot ± standard error in 2013, 2014, and 2015. Colours indicate the forest type and leaf litter treatment. Significant categorical variables were followed by a Tukey HSD test based on growth between 2013 and 2015 (α=0.05).

A2.2.4 Comparison of different moss measuring methods

Correlations among the three colour measurement methods employed were low ($r^2 < 0.33$; Figure A2.5). Colour was also weakly correlated to the sampling unit level measurements of moss canopy height ($r^2 < 0.38$; Figure A2.4). I found that visual estimate of the percent cover of green *H. splendens* was the colour variable that correlated the best with individual moss growth variables ($r^2 = 0.38$-0.54), followed by DGCI ($r^2 = 0.36$-0.50) and then by NDVI ($r^2 = 0.15$-0.35; Figure A2.5).

Surprisingly, there was no correlation between moss growth estimated through sampling unit height from the forest floor and through the cranked wire method ($r^2 = 0.09$; Figure A2.5). Both methods were, however, moderately correlated to the more precise measures of individual moss growth ($r^2 = 0.19$-0.43), particularly moss biomass and area (Figure A2.5). It is surprising that these variables were not as strongly related to the number of segments, as the production of new segments should be an important mechanism in increasing the height of the moss canopy.

Among the individual measurements taken on the marked moss individuals, the strongest correlations were found between the total moss measurements and the measurements taken on the dominant chain of segments ($r^2 = 0.71$-0.97; Figure A2.5). This suggests that measuring all moss segments, especially in an area where side branches are common like Alaska (Chapter 3), would give a more precise estimate of moss growth, but focusing only on the dominant chain of steps would give similar results. Moss biomass and area were very strongly related ($r^2 = 0.87$-0.97; Figure A2.5), which is consistent with the fact that moss area was the main predictor used in the allometric equations to estimate moss biomass in 2013 (Appendix 3). The 2015 data of moss biomass was directly measured, and not obtained through the allometric equations. Counts of the number of segment per moss shoot were also correlated with biomass and area ($r^2 = 0.60$-0.71; Figure A2.5).

Color
Sampling unit level measures of height
Individual moss measurements
2015 green H.splendens (% cover)
2015 Dark Green Color Index (DGCI)
2015 Normalized Difference Vegetation Index (NDVI)
Sampling unit height from the forest floor (cm)
Distance of the moss canopy from the cranked wire (cm)
BiomassALL
BiomassSingle
AreaALL
AreaSingle
StepALL
StepSingle
r2=0.33 p=0.0002
r2=0.14 p=0.1406
r2=-0.06 p=0.5316
r2=0.38 p>0.0001
r2=0.29 p=0.0016
r2=-0.02 p=0.8288
r2=0.18 p=0.0439
r2=0.09 p=0.3310
r2=0.33 p=0.0002
r2=0.09 p=0.3266
r2=0.54 p<0.0001
r2=0.38 p>0.0001
r2=0.26 p=0.0040
r2=0.43 p<0.0001
r2=0.38 p>0.0001
r2=0.50 p<0.0001
r2=0.36 p<0.0001
r2=0.29 p<0.0011
r2=0.40 p<0.0001
r2=0.42 p<0.0001
r2=0.97 p<0.0001
r2=0.54 p<0.0001
r2=0.46 p<0.0001
r2=0.33 p=0.0003
r2=0.35 p<0.0001
r2=0.40 p<0.0001
r2=0.93 p<0.0001
r2=0.91 p<0.0001
r2=0.49 p<0.0001
r2=0.44 p<0.0001
r2=0.35 p=0.0001
r2=0.30 p=0.0009
r2=0.43 p<0.0001
r2=0.87 p<0.0001
r2=0.91 p<0.0001
r2=0.97 p<0.0001
r2=0.49 p<0.0001
r2=0.40 p<0.0001
r2=0.15 p=0.0946
r2=0.40 p<0.0001
r2=0.19 p=0.0350
r2=0.69 p<0.0001
r2=0.60 p<0.0001
r2=0.68 p<0.0001
r2=0.57 p<0.0001
r2=0.38 p<0.0001
r2=0.50 p<0.0001
r2=0.23 p=0.0112
r2=0.34 p=0.0001
r2=0.28 p=0.0020
r2=0.60 p<0.0001
r2=0.65 p<0.0001
r2=0.65 p<0.0001
r2=0.70 p<0.0001
r2=0.71 p<0.0001
r² < 0.2
r² 0.2, 0.5
r² < 0.5, 0.7
r² < 0.7, 0.9
r² > 0.9
Alaska paper birch
Addition
Ambient
Control
Exclusion
Black spruce
Addition
Ambient
Control
Exclusion

Figure A2.5: (previous page) Correlations between all the moss measurements used. Colour measurements are the 2015 values, while all the growth measurements correspond to the difference between 2015 and 2013. Upper panels represent all the sampling units as points, and the colours represent forest type and leaf litter treatments. Lower panels present the correlation (r^2) and *p* values. Shadings represent the strength of the correlations, with grey shading indicating a stronger relationship. DGCI: Dark Green Colour Index. NDVI: Normalized Difference Vegetation index.

A2.3 Discussion

A2.3.1 Species composition

Even if the results of the multivariate analysis were highly significant, patterns of change in species composition according to leaf litter treatment were difficult to interpret. I hypothesize that the major changes are likely due to a reduction in the cover of green *H. splendens* in the SUs subjected to experimental and ambient leaf litter inputs. This is consistent with the results from Chapter 3, as well as with the results from section A2.2.3, where I found that growth of *H. splendens* was reduced by leaf litter. A small increase in the abundance of the acrocarpous *Polytrichum commune* or *Peltigera aphtosa* associated with leaf litter is more likely to be related to the slight sinking down of the dominant pleurocarpous feather mosses rather than a colonization event. There may have been more changes in the abundance of small inconspicuous bryophyte taxa in the transplants (e.g. leafy liverworts), but those were not recorded because of time and taxonomical resolution constraints. Decomposition of dead moss tissue (Cornelissen et al. 2007) and moss colonization, particularly when no mineral soil or bare ground is exposed (Chapter 2), are very slow processes. I therefore suppose that three years were not sufficient to really observe important and obvious changes in moss communities.

A2.3.2 Colour

Differences in moss greenness among the various treatments were a very striking feature in the field, with mosses under leaf litter being very brown and mosses without leaf litter to be a healthy looking yellowish green. However, quantifying moss greenness was a challenge. Overall, all results pointed towards a high leaf litter cover leading to a reduction in the greenness of the mosses, while exclusion treatments or low amount of leaf litter cover lead to mosses that were greener. There was no clear effect of forest type. None of the colour indices were very well correlated to the data measured on the tagged individuals, except for the visual estimates of moss greenness. The difference in moss colour between treatments with leaf litter and without was subjectively striking in the field, but were nevertheless associated with inconsistent results of the moss colour indices.

I obtained some interesting results using the DGCI methods, with an optimum moss greenness at low covers of leaf litter. This method has some flaws that could be improved in future studies. For example, results could be affected by camera sensors, as well as ambient light

conditions. Using a single camera to capture all the pictures would improve the accuracy, as well as including a calibration colour square to adjust the white balance in all the pictures. I also noticed that the DGCI, a method that was created to estimate vascular plant greenness (Karcher and Richardson 2003), may not be totally appropriate for estimating bryophyte greenness. Feather mosses like *H. splendens* tend to be shades of green that are lighter and yellower and have stems that are reddish, which is not well captured by such an index. Developing a greenness index for feather mosses that would correlate well with moss growth could therefore be an interesting venue in future studies.

NDVI did not seem to be a very good indicator of changes in moss growth and biomass accumulation. I hypothesize that this is probably due to some methodological issues. The camera used to get NDVI values is meant to be used with no shadows or canopy cover, and under a constant lighting, which was not the case under deciduous and coniferous forest covers. I tried to use the camera only on apparently constant overcast days, but because of time constraints this was not always possible. Moss colour can be affected by its moisture content, and NDVI measurements particularly very sensitive to moisture (Arkimaa et al. 2009). Taken as a whole, this indicates that NDVI may therefore not be the most appropriate for quantifying small scale moss greenness.

This far, visual estimates of green moss cover appear to be the best of the tested methods. Visual estimates gave results more similar to the moss growth measures, and were a very cost-effective method. Its accuracy may be improved if the observer used calibration colours. DGCI is another interesting venue, as it is the fastest method in the field and was also relatively cheap, requiring only a digital camera. NDVI does not seem to be a very good indicator of moss greenness under the conditions in which I used it.

A2.3.3 Moss growth

Measurements of moss biomass and area are assumed to be the most precise estimates of moss growth out of all the variables that were used since these estimate were taken directly on the moss individuals, but also the most time consuming. It seems like following moss growth only on the dominant chain of segments is a good estimate of total moss growth and may be easier and faster to track in the field. Moss area and biomass were very strongly correlated and either can probably be used to estimate the other. The count of moss segments was not as precise

to relate to moss biomass increases, but it is a method that can be done a lot faster than measuring each segment. Including a count of lateral branches may be a way to improve the accuracy of the segment count metric, as the expansion of side branches likely increase weight more than the production of a single-stemmed segment. Depending on the level of precision required by a study, all of those measurements could be appropriate, given that a subsample is harvested for calibration.

Sampling unit height from the forest floor and moss cranked wire measurements were rough estimates of moss growth, but these measurements were a lot faster to take in the field. Using these methods, I was able to track the relative impact of the leaf litter treatments on moss growth, but some calibration and destructive harvest would be needed if these values were to be converted in moss biomass. These measurements also tended to record changes in the structure of the moss mat (collapse following transplantation or leaf litter addition) due to the lack of lateral stability or hydraulic isolation. This latter effect was likely particularly important in birch stands and may explain the decrease in growth of the exclusion treatment as measured by the cranked wire. In the field, I observed that this lack of stability lead to a form of lateral flattening of the transplants and an apparent lowering of shoot density, rather than an actual decrease in moss growth. Alternatively, transplants in spruce stands that rested on decomposing moss had a higher potential for "sinking" than transplants in birch stands that rested on mineral soil, as seemed to be the case when comparing height from the forest floor of the leaf litter addition transplants in both forest types. This dramatic decline in moss growth under leaf litter addition in spruce stands was not observed in any of the other measurements used.

A2.4 References

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APPENDIX 3: ALLOMETRIC EQUATIONS USED TO ESTIMATE *HYLOCOMIUM SPLENDENS* BIOMASS

A3.1 Methods

Sampling took place at the sites that were surveyed in Chapters 3 and 4. In September 2013, June 2014, and September 2014, I collected one core (100 cm^2) of *Hylocomium splendens* (Hewd) Schimp. in the proximity of each plot from each block in black spruce (*Picea mariana* Mill. B.S.P.) and Alaska paper birch stands (*Betula neoalaskana* Sarg.). The core was taken to the laboratory where I measured moss shoot density per core, and recorded dimensions (length, width and weight) of moss segments from 5-20 moss individuals per sample. Area of moss segments was calculated as (width*length)/2. Each moss segment was attributed a segment number, with 1 indicating the most recent segments (see Figure 3.2), as well as letters when multiple growing points were present in the moss sample. Segments were measured down to the eighth segment from the top. However, I restricted the analysis to the top five segments in order to have a sufficient sample size. This number also corresponds to what was measured in Chapter 3. Moss segment colour (Dignard et al. 2013), branching pattern (single, pinnate or bipinnate), number of sporophytes, and presence of a bud were also recorded.

I used mixed models to build allometric equations allowing prediction of moss biomass per segment using moss dimensions, as well as some of the variables measured on the moss samples. Following initial investigation of the data, I determined that sampling month and year did not affect the allometric equations (data not shown). Since moss segment colour, branching patterns, sporophytes, and bud presence were not recorded in the field measurements of the moss transplant experiment (Chapter 3), these variables were not included in the building of the allometric equations. I employed a model selection approach based on Akaike's Information Criterion (AIC) (Crawley 2007) on linear mixed effects models using the library *lmerTest* (Kuznetsova et al. 2015). The full model included an interaction between segment number and moss area per shoot, as well as between forest type and sampling block. The random effects included a random slope according to moss area as I suppose that the relationship between moss area and biomass may vary according to individuals and patches, as well as a random intercept accounting for the identity of each moss sample nested within each moss core (Zuur et al. 2009). Moss biomass and area were transformed using the natural logarithm to meet normality and homoscedasticity assumptions. The accuracy of the allometric equation was tested through a

Pearson correlation using the predicted and measured moss segment biomass from the August 2015 harvest from the moss transplant experiment (Chapter 3).

A3.2 Results

Three models were selected as being within 2 delta AICc values (Table A3.1). The inclusion of forest type and block to the model did not improve it significantly. For parsimony, I therefore selected the simplest of the models, model 4, to estimate moss segment biomass in all the sampling units from the moss transplant experiment on the basis of segment area and number (Table A3.2). The interaction between moss segment and area came mostly from the much steeper slope associated with mosses in the first segment (new shoots) than for the older four other segments (Figure A3.1). Predicted values estimated using this model were compared against the measured moss biomass values of August 2015. My model accurately predicted the 2015 moss biomass per segment ($r^2 = 0.9707$, $p < 0.0001$; Figure A3.2).

Table A3.1: Results from model selection using the delta AICc criterion. Models 1, 3, and 4 were selected as being the best ones.

	Model #	**dAICc**	**df**
Ln(Area+1)*Segment+Forest type	3	0	18
Ln(Area+1)*Segment	4	0.3	17
Ln(Area+1)*Segment+Forest type*block	1	1.5	22
Ln(Area+1)*Segment+Forest type+block	2	3.3	20
Null model	0	492.3	8

Table A4.3: Allometric equation model estimating moss biomass (natural logarithm transformed) using moss area (natural logarithm transformed) and segment number. The random effects included a random slope for moss area and a random intercept for moss shoot within moss patch.

	Estimate	**Standard error**	***t* value**	***p* value**
Intercept	0.575	0.022	26.136	< 0.0001
Ln(Area+1)	1.180	0.022	54.546	< 0.0001
Segment 2	0.285	0.032	8.865	< 0.0001
Segment 3	0.389	0.037	10.501	< 0.0001
Segment 4	0.353	0.040	8.833	< 0.0001
Segment 5	0.444	0.048	9.306	< 0.0001
Ln(Area+1)*Segment 2	-0.182	0.027	-6.686	< 0.0001
Ln(Area+1)*Segment 3	-0.248	0.029	-8.575	< 0.0001
Ln(Area+1)*Segment 4	-0.239	0.031	-7.701	< 0.0001
Ln(Area+1)*Segment 5	-0.277	0.036	-7.598	< 0.0001

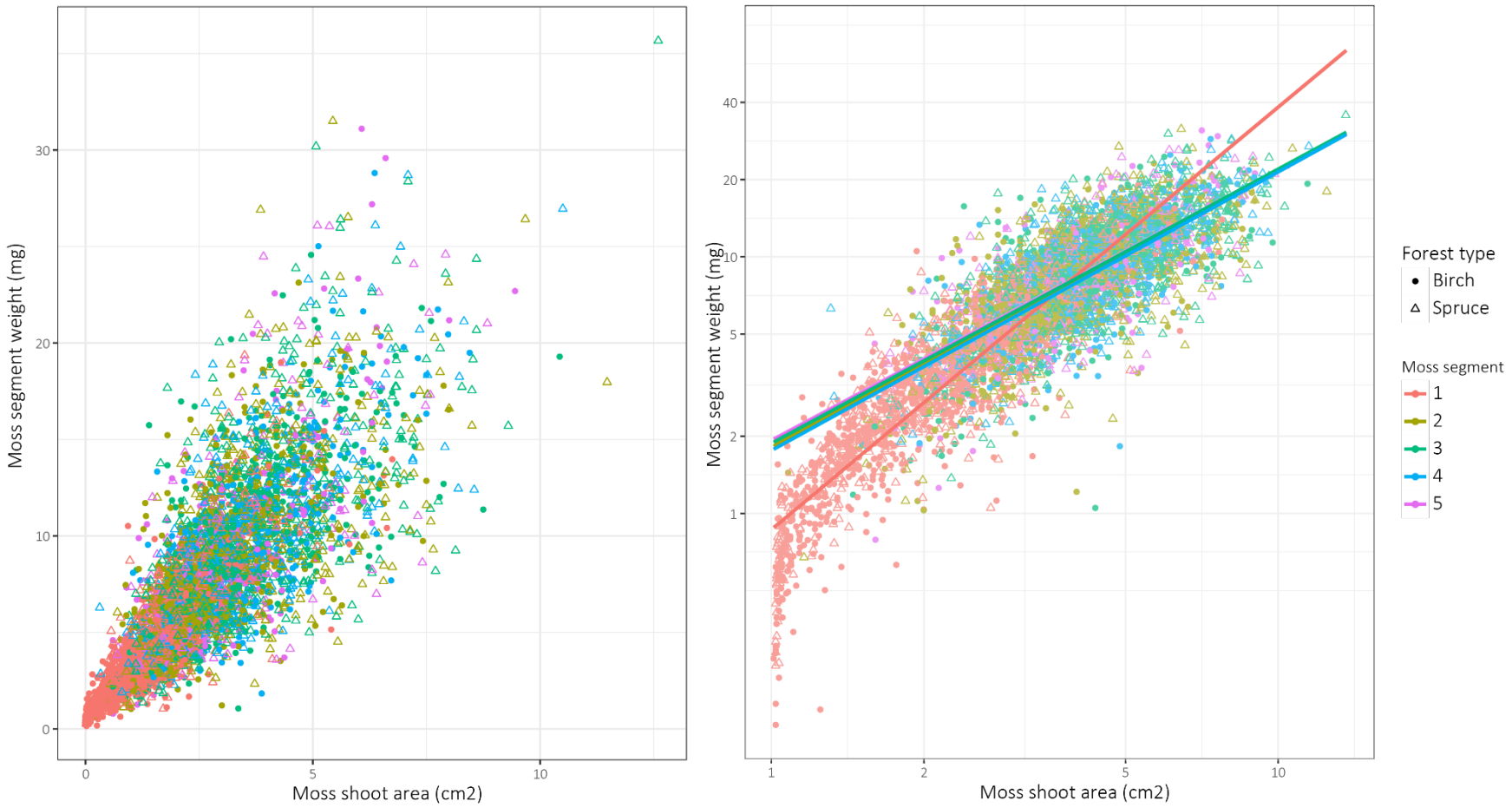

Figure A3.1: Moss shoot area (cm^2) and moss segment total biomass (mg) for samples of *Hylocomium splendens*. Each point represents a moss segment (n = 4681 segments from 851 moss shoots). Shapes represent forest type and colours represent moss segment number, with segment 1 being the newest. a) Raw data used to build allometric equations. b) Linear regressions for each moss segment to predict moss weight using transformed data. The y-axis on this panel is on a natural logarithm scale.

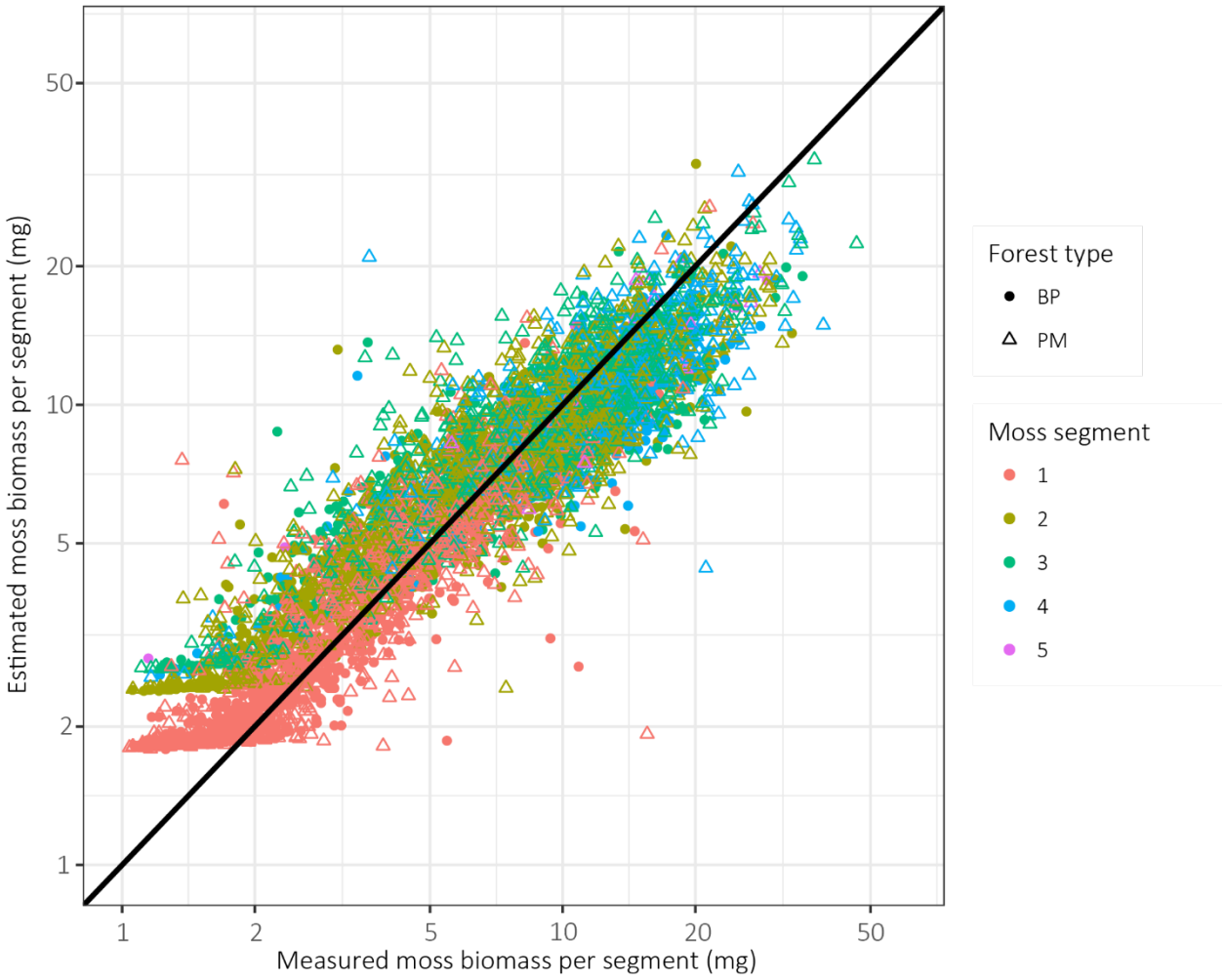

Figure A3.2: Comparison of measured moss biomass per segment (mg) and the predicted moss biomass per segment (mg) using the allometric equation from Table 2 (n = 4681 segments from 851 moss shoots). Moss biomass was truncated just below 2 mg, as this was associated with the weight of the smallest moss segments that I was able to dissect and measure. Data are samples harvested from the moss transplant experiment in August 2015. Shapes represent forest type and colours represent moss segment number, with segment 1 being the newest. The black line is the 1:1 ratio. Note that the y-axis is on a natural logarithm scale.

A3.3 References

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APPENDIX 4: SUPPORTING INFORMATION FOR CHAPTER 3

Table A4.1: Description of the studied stands. Site description was completed by Melvin et al. (2015).

	Betula neoalaskana				*Picea mariana*			
Block	A	B	C	Average	A	B	C	Average
Site description								
Slope	12.2	13.4	8.8	11.5	6.4	10.6	4.2	7.1
Aspect	42.0	67.4	106.2	71.9	18.0	48.4	87.0	51.1
Organic layer depth	7.0	7.2	8.6	7.6	16.2	18.4	14.5	16.4
GLA canopy cover	82.9	79.1	80.7	80.9	56.9	58.3	71.5	62.3
Average climate data								
Air temperature (°C)	13.6	13.7	12.9	13.4	13.6	13.8	12.2	13.2
Soil temperature (°C)	7.0	7.6	8.5	7.7	4.5	5.2	2.7	4.1
Photosynthetic active radiation (W m^{-2})	152.0	114.9	168.5	145.1	591.1	392.2	168.8	384.0
Relative air humidity (%)	69.3	70.5	71.5	70.4	70.0	71.8	75.9	72.5
Soil moisture (m^3 m^{-3})	0.1	0.2	0.1	0.1	0.2	0.2	0.2	0.2
Species cover (%)								
Hylocomium splendens	7.3	12.6	1.8	7.2	58.3	52.8	47.0	52.7
Pleurozium schreberi	2.8	4.8	1.4	3.0	58.9	56.6	38.9	51.4
Dicranum spp.	0.1	0.5	0.8	0.5	0.0	0.3	2.4	0.9
Polytrichum spp.	0.3	0.5	0.1	0.3	23.4	24.8	6.4	18.2
Aulacomnium spp.	0.1	0.4	0.0	0.2	3.3	1.4	9.3	4.6
Sphagnum spp.	0.1	0.0	0.0	0.0	14.1	10.6	5.3	10.0
Ptilium crista-castrensis	0.0	0.0	0.0	0.0	0.6	0.0	1.9	0.8
Peltigera aphthosa	0.0	0.3	0.0	0.1	6.4	2.5	1.0	3.3
Peltigera spp.	0.0	0.0	0.0	0.0	1.6	0.5	0.0	0.7
Leaf litter	95.0	93.6	97.8	95.5	38.5	53.8	57.8	50.0

Table A5.2: Analysis of Variance Table of type III with Satterthwaite approximation for degrees of freedom for a linear mixed-model[a] comparing the average weight of the 2012 step per transplant according to treatment and forest type (data not transformed).

	Sum of squares	**Mean squares**	**DFn, DFd**	**F value**	***p* value**
Forest type	3.111	3.111	1, 110	0.248	0.6193
Treatment	19.497	6.499	3, 110	0.519	0.6703
Forest type*Treatment	50.996	16.999	3, 110	1.357	0.2600

[a] Model code: lmer(2012 weight ~ Forest type*Treatment + (1|Block/Forest type/Plot)). Model conducted using the *lmerTest* (Kuznetsova et al. 2015) and *lme4* (Bates et al. 2014) packages.

Table A4.3: Results of a binomial regression model with a log link function[a] comparing the average leaf litter cover on the SUs in 2012 to 2015 among forest types and treatments, and a forest type × treatment interaction. Procedural control is the baseline treatment. The significant effects are shown in bold font.

	Estimate	**Standard error**	***z* value**	***p* value**
Forest type black spruce	0.7409	0.0552	13.416	**< 0.0001**
Treatment addition	-2.0970	0.0845	-24.814	**< 0.0001**
Treatment ambient	2.0406	0.1232	16.559	**< 0.0001**
Treatment exclusion	0.8869	0.0889	9.973	**< 0.0001**
Forest type black spruce × Treatment addition	-4.1137	0.1544	-26.639	**< 0.0001**
Forest type black spruce × Treatment ambient	1.9527	0.1731	11.278	**< 0.0001**
Forest type black spruce × Treatment exclusion	-1.1226	0.1294	-8.675	**< 0.0001**

[a] Model code: glm(Leaf litter cover / Total SU area ~ Forest type*Treatment, family=binomial, weights= Total SU area). Model conducted using the *lme4* package (Bates et al. 2014).

Table A4.4: Analysis of variance tables of type III with Satterthwaite approximation for degrees of freedom for a linear mixed-model with repeated measures model comparing average moss shoot biomass (total) according to forest type, leaf litter treatment and time. Data were log transformed and a Bonferoni correction was applied for subtables b-d, with a critical *p* value of 0.017. Significant effects are shown in bold font. Models are broken up based on the time period examined: a) full model (2013-2015); b) data from September 2013; c) data from September 2014; d) data from August 2015.

	Sum of squares	Mean squares	DFn, DFd	F value	*p* value
a) Full model (2013-2015)[a]					
Forest type	0.620	0.620	1, 26	27.060	**<0.0001**
Treatment	0.952	0.317	3, 84	14.850	**<0.0001**
Sampling time	85.663	42.831	2, 224	1869.870	**<0.0001**
Forest type*Treatment	0.156	0.052	3, 84	2.280	0.0696
Forest type* Sampling time	0.145	0.073	2, 224	3.400	**0.0349**
Treatment*Sampling time	3.079	0.513	6, 224	24.000	**<0.0001**
Forest type*Treatment*Sampling time	0.785	0.131	6, 224	6.120	<0.0001
b) September 2013[b]					
Forest type	0.735	0.735	1, 26	25.794	**<0.0001**
Treatment	0.166	0.055	3, 84	1.941	0.1292
Forest type*Treatment	0.177	0.060	3, 84	2.074	0.1098
c) September 2014[b]					
Forest type	1.204	1.205	1, 26	26.904	**<0.0001**
Treatment	1.406	0.469	3, 84	10.471	**<0.0001**
Forest type*Treatment	0.373	0.124	3, 84	2.779	0.04613
d) August 2015[b]					
Forest type	1.526	1.526	1, 26	18.329	**0.0002**
Treatment	6.484	2.161	3, 84	25.961	**< 0.0001**
Forest type*Treatment	1.052	0.351	3, 84	4.212	**0.0079**

[a] Model code: lmer(Moss biomass ~ Forest type*Treatment*Sampling time + (1|Block/Forest type/Plot/Sampling unit)). Model conducted using the *lmerTest* (Kuznetsova et al. 2015) and *lme4* (Bates et al. 2014) packages.
[b] Model code: lmer(Moss biomass ~ Forest type*Treatment + (1 |Block/Forest type/Plot)). Model conducted using the *lmerTest* (Kuznetsova et al. 2015) and *lme4* (Bates et al. 2014) packages.

Table A4.5: Analysis of variance tables of type III with Satterthwaite approximation for degrees of freedom from the best linear mixed effect model[a] relating a) moss growth between June 2013 and August 2015 and b) change in number of segments between June 2013 and August 2015 to leaf litter cover, canopy cover, and forest type. Significant effects are shown in bold font.

	Sum of squares	Mean squares	DFn, DFd	F value	*p* value
a) Moss growth					
Leaf litter cover	4204.0	4204.0	1, 91.3	77.782	**<0.0001**
Canopy cover	156.5	156.5	1, 81.7	2.896	0.0926
Forest type	240.7	240.7	1, 82.0	4.453	**0.0379**
Canopy cover*Forest type	305.3	305.3	1, 82.7	5.649	**0.0198**
b) Number of segments					
Leaf litter cover	16.1954	16.1954	1, 113.0	27.7350	**<0.0001**
Canopy cover	0.6350	0.6350	1, 114.6	0.9698	0.3268
Forest type	2.0308	2.0308	1, 114.1	3.1016	0.0809
Canopy cover*Forest type	2.2072	2.2072	1, 114.0	3.3710	0.0690

[a] Final model code: lmer(response variable ~ Leaf litter cover + Canopy cover + Forest type+ Canopy cover*Forest type + (1|Block/Forest type/Plot)). The initial model in both cases included the three-way interaction between leaf litter cover, canopy cover, and forest type. Model conducted using the *lmerTest* (Kuznetsova et al**.** 2015) and *lme4* (Bates et al. 2014) packages.

Table A4.6: Analysis of Variance Table of type III with Satterthwaite approximation for degrees of freedom for a linear mixed-model comparing a) moss greenness in September 2012[a] and b) moss greenness in August 2015[b] according to forest type (spruce vs. birch), treatments (procedural control, ambient leaf litter, litter addition, and litter exclusion), and a forest type × treatment interaction. Moss greenness is represented as a modified dark-green colour index (DGCI). Significant effects are shown in bold font, and different letters indicate that the treatments were significantly different (α=0.05) following a Tukey HSD posthoc test.

	Sum of squares	**Mean squares**	**DFn, DFd**	**F value**	***p* value**	Post-hoc test results
a) 2012						
Forest type	0.0446	0.0446	1, 4	4.7556	0.0947	
Treatment	0.2442	0.0814	1, 84	8.6875	**<0.0001**	
Procedural control						a
Addition						ab
Ambient						b
Exclusion						a
Forest type*Treatment	0.0622	0.0207	1, 84	2.2120	0.0927	
b) 2015						
Forest type	0.0025	0.0025	1, 5.8	0.4300	0.5371	
Treatment	0.2489	0.0830	3, 109.4	14.1457	**<0.0001**	
Procedural control						b
Addition						a
Ambient						a
Exclusion						b
Forest type*Treatment	0.0289	0.0287	3, 108.2	1.6447	0.1833	
2012 moss greenness (DGCI)	0.0286	0.0286	1, 24.3	4.8817	**0.0368**	

[a] Model code: lmer(2012 moss greenness ~ Forest type*Treatment + (1|Block/Forest type/Plot)). Model conducted using the *lmerTest* (Kuznetsova et al. 2015) and *lme4* (Bates et al. 2014) packages.
[b] Model code: lmer(2015 moss greenness ~ Forest type*Treatment+ 2012 moss greenness + (1|Block/Forest type/Plot)). Model conducted using the *lmerTest* (Kuznetsova et al. 2015) and *lme4* (Bates et al. 2014) packages.

Table A4.7: Analysis of Variance Table of type III with Satterthwaite approximation for degrees of freedom for a linear mixed-model[a] comparing moss greenness in 2015 (DGCI) according to leaf litter cover (%), the quadratic term of leaf litter cover, canopy cover (%), forest type, and moss greenness in 2012. Numerical predictors were scaled prior to analysis. Significant effects are shown in bold font.

	Sum of squares	**Mean squares**	**DFn, DFd**	**F value**	***p* value**
Leaf litter cover (%)	0.0922	0.092181	1, 109.0	18.1386	**<0.0001**
Leaf litter cover (%) ^ 2	0.0414	0.0414	1, 110.1	8.1425	**0.0052**
Forest type	0.0162	0.0162	1, 110.1	3.1921	0.0767
Canopy cover (%)	<0.0001	<0.0001	1, 111.0	0.0005	0.9815
Leaf litter*Forest type	0.0024	0.0024	1, 109.4	0.4712	0.4939
Leaf litter*Canopy cover (%)	0.0037	0.0037	1, 109.6	0.7198	0.3981
Forest type*Canopy cover (%)	0.0111	0.0111	1, 111.0	2.1889	0.1418
2012 moss greenness (DGCI)	<0.0001	<0.0001	1, 97.5	0.0058	0.9396

[a] Model code: lmer(2015 DGCI ~ Leaf litter cover*Forest type + (Leaf litter cover^2) + 2012 DGCI + (1|Block/Forest type/Plot). Model conducted using the *lmerTest* (Kuznetsova et al. 2015) and *lme4* (Bates et al. 2014) packages.

Table A4.8: Results from the GLM with zero-inflated Poisson, comparing the total number of sporophytes on the segments after the second year (> yellow; Figure 3.2) among procedural controls between black spruce and paper birch stands.

	Estimate	Std. Error	z value	Pr(>|z|)
a) Procedural controls[a]				
(Intercept)	2.326	2.53	0.92	0.3580
Forest type (spruce)	2.787	1.427	1.95	0.0510
Number of moss shoots included	-0.376	0.223	-1.68	0.0930
b) Transplants[b]				
(Intercept)	-2.405	1.574	-1.53	0.1264
Forest type (spruce)	1.714	0.548	3.13	0.0018
Leaf litter cover	-0.036	0.008	-4.63	>0.0001
Number of moss shoots included	0.226	0.143	1.58	0.1150

[a] Code for selected model: glmmadmb(Sporophyte count ~ Forest type + Number of moss shoots + (1|Block/Forest type), zeroInflation=TRUE, family="poisson"). Random effect of Block:Forest type accounts for 0.992 ± 0.996 (standard deviation (SD)) in the variance of the intercept. The zero-inflation factor is 0.553 ± 0.146 (SD). Model conducted using the *glmmadmb* package (Skaug et al. 2014).

[b] Code for selected model: glmmadmb(Sporophyte count ~ Forest type + Leaf litter cover + Number of moss shoots + (1|Block/Forest type), zeroInflation=TRUE, family="poisson"). The random effects of Block accounts for 0.00004 ± 0.0066 (SD), Block:Forest type accounts for 0.000002 ± 0.0015 (SD), and Block:Forest type:UniquePlotID accounts for 0.5429 ± 0.7368 (SD) in the variance of the intercept. The zero-inflation factor is 0.374 ± 0.103 (SD). Model conducted using the *glmmadmb* package (Skaug et al. 2014).

Table A4.9: Analysis of variance table of type III with Satterthwaite approximation for degrees of freedom for a linear mixed-model repeated measures model[a] comparing average number of segment per moss shoot per transplant according to forest type, litter treatment and time. Data were square root transformed. Significant effects are shown in bold font.

	Sum of squares	**Mean squares**	**DFn, DFd**	**F value**	***p* value**
Forest type	0.072	0.0722	1, 2	6.18	0.1308
Treatment	0.462	0.1541	3, 84	13.19	**<0.0001**
Sampling time	32.152	16.0759	2, 224	1375.27	**<0.0001**
Forest type * Treatment	0.083	0.0276	3, 84	2.36	0.0774
Forest type * Sampling time	0.051	0.0255	2, 224	2.18	0.1152
Treatment * Sampling time	0.595	0.0992	6, 224	8.49	**<0.0001**
Forest type * Treatment * Sampling time	0.116	0.0193	6, 224	1.65	0.1350

[a] Model code for a): lmer(Moss segments ~ Forest type*Treatment*Sampling time + (1|Block/Forest type/Plot/Sampling unit)). Model conducted using the *lmerTest* (Kuznetsova et al. 2015) and *lme4* (Bates et al. 2014) packages.

Table A4.10: Percentages of moss shoots that produced more than one segment in a year along the main axis of growth according to forest types and leaf litter treatments.

Forest type	Treatment	Samples that produced more than 1 segment (%)	
		2013-2014	2014-2015
Birch		**7.9**	**21.0**
	Litter addition	5.5	13.0
	Ambient litter	6.1	12.2
	Procedural control	8.2	32.4
	Litter exclusion	11.8	26.6
Spruce		**39.5**	**28.6**
	Litter addition	33.3	15.5
	Ambient litter	36.4	30.1
	Procedural control	39.7	36.8
	Litter exclusion	49.5	33.1
	Average	**22.0**	**24.7**

Table A4.11: Results of mixed-effect logistic regression models[a] of proportion of moss biomass allocated to new segments in 2014-2015 analyzed with leaf litter treatments and forest types, and a forest type × treatment interaction. Procedural control is the baseline treatment. The significant effect is shown in bold font.

	Estimate	**Standard error**	***z* value**	***p* value**
Forest type black spruce	-0.3536	0.2978	-1.187	0.2351
Treatment addition	-0.8139	0.4017	-2.026	**0.0428**
Treatment ambient	-0.6236	0.3364	-1.854	0.0638
Treatment exclusion	-0.0067	0.2713	-0.025	0.9802
Forest type black spruce × Treatment addition	0.2159	0.5560	0.388	0.6978
Forest type black spruce × Treatment ambient	0.4212	0.4296	0.981	0.3268
Forest type black spruce × Treatment exclusion	0.0994	0.3742	0.266	0.7906

[a] Model code: glmer(New segment growth / Total growth ~ Forest type*Treatment + (1|Block/Forest type/PlotID), family=binomial,weights= Total growth). Model conducted using the *lme4* package (Bates et al. 2014).

Table A4.12: Results of linear mixed-effect models[a] of a) *Hylocomium splendens* (mg per shoot, square root transformed) and b) *Pleurozium schreberi* growth (mg per shoot, natural logarithm transformed) compared among treatments (control, litter addition, leaf litter exclusion, and leached leaf litter), and using initial moss weight as a covariate (estimated from the measurements taken in September 2014 (a) or June 2015(b)). Significant effects are shown in bold font.

	Sum of squares	Mean squares	DFn, DFd	F value	*p* value
a) *Hylocomium splendens*					
Treatment	5.1515	1.7172	3, 11.7	1.6449	0.2324
Initial moss weight (mg)	5.6847	5.6847	1, 77.5	5.4453	**0.0222**
b) *Pleurozium schreberi*					
Treatment	2.0561	0.6854	3, 12.5	7.6439	**0.0037**
Initial moss weight (mg)	2.3425	2.3425	1, 74.8	26.1261	**<0.0001**

[a] Model code: lmer(Moss biomass ~ Treatment + Initial moss weight + (1 |Plot)). Model conducted using the *lmerTest* (Kuznetsova et al. 2015) and *lme4* (Bates et al. 2014) packages.

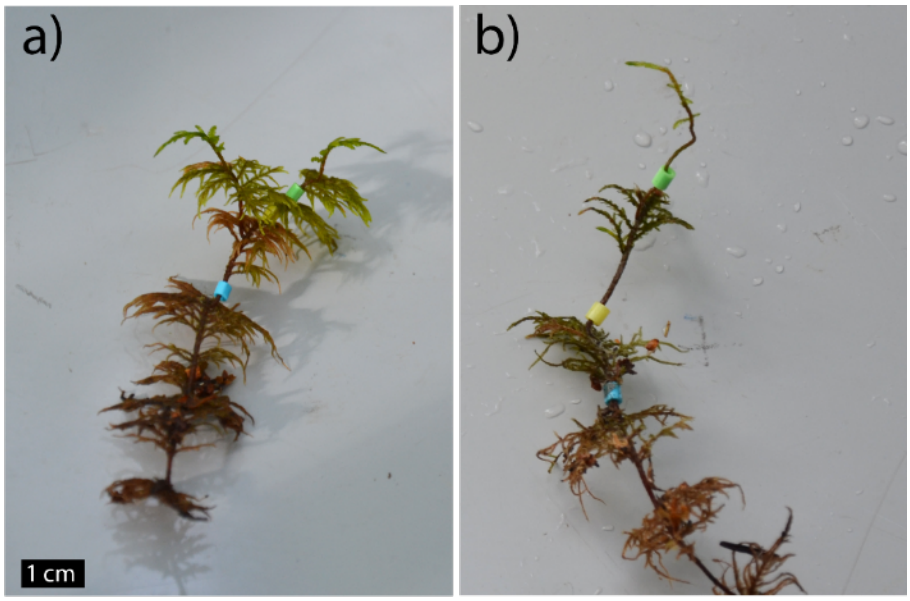

Figure A4.1: Examples of the altered growth form of mosses found in the leaf litter addition treatments vs. a more typical growth form. a) Sample from the birch stand in block B, plot 5, exclusion treatment (sample HI23). b) Sample from the birch stand in block C, plot 5, addition treatment (sample D8). Pictures by M. Jean.

APPENDIX 5: SUPPORTING INFORMATION FOR CHAPTER 4

Table A5.1 Calculation of N_2-fixation rates using the natural abundance and enriched moss samples.

1. Calculate the ratios (R) values at times 0 (natural abundance samples) and 1 (enriched samples). 0.0037137 is the ratio of $^{15}N/^{14}N$ in the standard (air).

$$Rt_0 = (0.0037137*(\delta 15N_0/1000))+0.0037137$$
$$Rt_1 = (0.0037137*(\delta 15N_1/1000))+0.0037137$$

2. Calculate the atom percent at time 0 (natural abundance) and 1 (enriched sample).

$$R_{T0}\ atom\% = (Rt_0/(1+Rt_0))*100$$
$$R_{T1}\ atom\% = (Rt_1/(1+Rt_1))*100$$

3. Calculate Delta F (or APE), i.e. change in the fraction enriched, also known as atom percent enrichment of the samples.

$$Delta\ F = T_1\ atom\% - T_0\ atom\%$$

4. Calculate the amount of ^{15}N fixed (mg) per g of moss dry mass. This is obtained by multiplying Delta F by the fraction of moss biomass that is N.

$$mg\ ^{15}N\ fixed/g\ dry\ mass = Delta\ F*(\%N/10)$$

5. Calculate the air volume in the headspace of the syringe volume by subtracting ibutton volume if applicable (0.048 ml), moss volume (1.17 ml for *Hylocomium splendens* and 1.00 ml for *Pleurozium schreberi*), ^{15}N tracer volume added (~10 ml) to the syringe volume (~20 ml).

$$Air\ in\ headspace\ (ml) = Syringe\ vol\ (ml) - (ibutton\ (ml)+moss) - {}^{15}N\ tracer\ added\ (ml)$$

6. Calculate the headspace air volume occupied by natural abundance N_2 gas (NAN_2). The percent N_2 in air is 78%.

$$Headspace\ NAN_2 = Air\ in\ headspace\ (ml) * (78/100)$$

7. Calculate the headspace air volume occupied by the $^{15}N_2$ tracer (ml). ^{15}N tracer volume added (~10 ml) and percent N in the $^{15}N_2$ tracer is 98%.

$$Headspace\ air\ volume\ occupied\ by\ {}^{15}N_2\ (ml) = {}^{15}N_2\ tracer\ vol\ added\ (ml) *(98\%\ {}^{15}N_2/100)$$

8. Calculate the ratio of air to tracer $^{15}N_2$ in the syringe headspace.

$$Headspace\ Air{:}tracer\ ratio = Headspace\ air\ volume\ occupied\ by\ NAN_2\ (ml) / Headspace\ air\ volume\ occupied\ by\ {}^{15}N_2\ (ml)$$

9. Scaling factor to convert ^{15}N fixed to $^{14}N + {}^{15}N$ fixed

^{15}N to air scaling = (1/(Headspace air volume occupied by $^{15}N_2$ (ml))/ (Headspace air volume occupied by $^{15}N_2$ + Headspace air volume occupied by NAN_2)

10. Mass of N fixed (μg) per unit moss dry mass per day (24 hours). Use ^{15}N to air scaling obtained at step 9, and ^{15}N fixed/g dry mass (mg) obtained at step 4. If the baseline value of $\delta^{15}N$ exceeded that of an enriched sample once a fixation rate of 0 was attributed to that sample.

Fixation rate (μg N fixed/g dry mass/day) = ^{15}N to air scaling * ^{15}N fixed/g dry mass (mg) *1000

Table A5.2 Detailed calculations for black spruce and Alaska paper birch annual nitrogen (N) budgets. With the exception of percentages, all values are presented in g m^{-2} $year^{-1}$. NPP: net primary productivity.

		Black spruce	Alaska paper birch
Plant N requirements	**Tree N requirements**[a]		
	N allocation to aboveground tree production		
	NPP_{tree}	192.5	463.9
	Crown growth (foliage and branches)	127.73	324.62
	% N in live foliage	1.08	2.52
	Stem growth (wood and bark)	64.76	139.29
	% N wood	0.10	0.14
	N loss in litter	-0.40	-2.00
	N_{AGtree}	2.08	11.69
	N allocation to fine roots		
	Fine root ANPP	216.95	166.72
	N_{fr}	1.52	1.60
	N_{tree}	**2.56**	**7.98**
	Net N in moss NPP[b]		
	Hylocomium splendens NPP	80.06	1.61
	H. splendens % N	0.64	1.04
	H. splendens N requirements	0.51	0.02
	Pleurozium schreberi NPP	36.38	0.89
	P. schreberi % N	0.52	0.93
	P. schreberi N requirements	0.19	0.01
	N_{moss}	**0.70**	**0.03**
	Total vegetation N requirements (N_{tree}+N_{moss})	**3.26**	**8.00**
N sources	**Daily N mineralization and nitrification**[c]		
	Daily N mineralization	0.01	0.05
	Total N mineralization and nitrification	2.15	9.18
	N_2-fixation vascular plants[d]	0.15	0.40
	Dry and wet N deposition[e]	0.03	0.03
	Total N inputs	**2.33**	**9.61**

[a] **Tree N requirements:** Measurements of tree net primary productivity (NPP_{tree}), percent N in foliage, and N losses through litter production (N_{lit}) were obtained from each forest type (black spruce and Alaska paper birch) and each block (A, B, and C) in my study area (Melvin et al. 2015). Percent N in wood was obtained from Alexander and Mack (2016). There were no significant differences among blocks (Melvin et al. (2015), and the values presented are the average for each forest type. Tree aboveground N requirement (N_{AGtree}) was estimated as the product of NPP and percent N in live foliage and wood. Allocation to fine roots is expected to be proportional to aboveground NPP. I used the ratio of N requirement for fine root production to tree NPP in black spruce (0.008) and birch/aspen (0.003) stands from Ruess et al. (1996) and applied these to estimates of NPP measured by Melvin et al. (2015) to estimate N allocation to fine root production at my sites (N_{fr}). Net tree N requirement (N_{tree}) was calculated as tree N_{AGtree}+ N_{fr} - N_{lit}.

[b] **N allocation to moss NPP:** Estimates of annual moss NPP were obtained for the two dominant feather moss species *Hylocomium splendens* and *Pleurozium schreberi* from Jean et al. (Chapter 3). Annual productivity was measured by re-measuring individual moss shoots from naturally occurring moss patches in the spruce and birch stands from the three blocks studied from 2013 to 2015 for *H. splendens*, and in the spruce stand of block A from

2014-2015 for *P. schreberi*. Annual tree N requirements were obtained by multiplying NPP by the percent N found in each moss species for each forest type using pooled data from all the samples in the current study.

[c] **N mineralization and nitrification:** Potential net N mineralization and nitrification rates were measured by Melvin et al. (2015) for each forest type (black spruce and Alaska paper birch) and block (A, B, and C). Average values per forest type are presented. The measurements were obtained following 90-day incubations in the laboratory of the fibric layer and top 10 cm of the mineral soil separately. I used the sum of both soil layers. Daily estimates were then multiplied by 180 days (growing season + shoulder season (Valentine et al. 2006)) in order to obtain yearly values that incorporate the growing season as well as shoulder seasons.

[d] **N_2-fixation:** Based on ecosystem mass accumulation of N in soils. The values came from lowland black spruce and upland birch stands near Fairbanks (Van Cleve et al. 1993). Because of the lack of methodological details, I used the values provided by Ruess et al. (1996) as a direct approximation.

[e] **Dry and wet N deposition:** Annual atmospheric N inputs from wet and dry N deposition obtained from Jones et al. (2005). Data were collected from the Poker Creek National Atmospheric Deposition Program (NADP) station (AK01) about 50 km Northeast of Fairbanks.

Table A5.3 ANOVA tables for inter-annual, intra-annual, and spatial comparisons of BNF (natural logarithm transformed) among forest types and moss species. Rates of N_2-fixation were log transformed prior to analysis to meet assumptions of normality and homoscedasticity.
a) Results of the four-way ANOVA model comparing N_2-fixation rates according to year, forest type, moss species and block with type III sum of squares for unbalanced design.

	Sum of squares	DFn	F value	*p*-value
Intercept	1.199	1	7.104	0.0086
Year	**52.81**	**2**	**156.40**	**< 0.0001**
Forest type	**11.42**	**1**	**67.64**	**< 0.0001**
Moss species	0.38	1	2.24	0.1369
Block	0.52	1	3.06	0.0825
Year × Forest type	**3.62**	**2**	**10.71**	**< 0.0001**
Year × Moss species	**2.41**	**2**	**7.15**	**0.0011**
Forest type × Moss species	0.16	1	0.94	0.3351
Year × Block	0.11	2	0.34	0.7158
Forest type × Block	**2.46**	**1**	**14.57**	**0.0002**
Moss species × Block	0.17	1	0.98	0.3234
Year × Forest type × Moss species	0.30	2	0.90	0.4103
Year × Forest type × Block	0.96	2	2.85	0.0608
Year × Moss species × Block	0.03	2	0.09	0.9107
Forest type × Moss species × Block	0.66	1	3.89	0.0505
Year × Forest type × Moss species × Block	0.68	2	2.02	0.1369
Residuals	24.82	147		

b) Results of the three-way mixed-effects model with type III Satterthwaite approximation for degrees of freedom comparing BNF (natural logarithm transformed) among forest types, moss species and month. Moss patches that were repeatedly sampled throughout the summer are the random effect, and account for 0.016 ± 0.100 (standard error) of the variance.

	Sum of squares	Mean squares	DFn	DFd	F value	*p*-value
Month	13.120	4.373	3	154	43.691	< 0.0001
Forest type	13.918	13.918	1	154	139.054	< 0.0001
Moss species	10.898	10.898	1	22	108.876	< 0.0001
Month × Forest type	0.887	0.296	3	154	2.953	0.0345
Month × Moss species	0.271	0.090	3	154	0.902	0.4416
Forest type × Moss species	1.195	1.1945	1	154	11.938	0.0007
Month × Forest type × Moss species	0.185	0.062	3	154	0.617	0.6050

c) Results of the three-way ANOVA model comparing BNF (natural logarithm transformed) rates according to forest type, moss species and block with type III sum of squares.

	Sum of squares	DF	F value	*p*-value
Intercept	156.392	1	1416.144	<0.0001
Forest Type	**3.043**	**1**	**27.554**	**<0.0001**
Block	**1.846**	**2**	**8.359**	**0.0004**
Moss Species	**2.765**	**1**	**25.036**	**<0.0001**
Forest Type × Block	**2.657**	**2**	**12.031**	**<0.0001**
Forest type × Moss species	0.068	1	0.614	0.4348
Block × Moss species	0.619	2	2.804	0.0642
Forest type × Block × Moss species	0.376	2	1.705	0.1858
Residuals	14.577	132		

Table A5.4 Model results for the structural equation model relating BNF rates (natural logarithm transformed) to environmental covariates using data from all forest types, moss species, year, months, and blocks.

Regressions		Estimate	Standard error[b]	Z-value	P-value	Standardized coefficient
Endogenous[a]	***Exogenous***[a]					
N_2-fixation rate	Precipitation[c]	0.477	0.037	13.015	<0.001	0.477
	Moss species[d]	0.353	0.081	4.375	<0.001	0.177
	Forest type[e]	1.46	0.192	7.61	<0.001	0.732
	Air temperature[f]	-0.385	0.036	-10.627	<0.001	-0.386
	Light availability[g]	-0.257	0.059	-4.328	<0.001	-0.258
	N mineralization[h]	0.419	0.089	4.721	<0.001	0.419
Air temperature	Forest type	0.020	0.107	0.189	0.850	0.010
Light availability	Forest type	1.526	0.068	22.471	<0.001	0.764
N mineralization	Forest type	-1.790	0.045	-39.566	<0.001	-0.896
Covariances		**Estimate**	**Standard error**	**Z-value**	**P-value**	**Standardized coefficient**
Air temperature	Precipitation	0.186	0.044	4.231	<0.001	0.186
	Light availability	0.148	0.024	6.157	<0.001	0.229
	N mineralization	0.143	0.041	3.49	<0.001	0.222
Light availability	Precipitation	-0.021	0.01	-2.227	0.026	-0.074
	N mineralization	-0.043	0.013	-3.336	0.001	-0.097
Moss species	Forest type	0.003	0.013	0.264	0.792	0.014

[a] Endogenous and exogenous variables represent the response and predictor variables, respectively, in a linear regression analysis.
[b] Standard errors obtained following a bootstrapping of 1000 iterations.
[c] Sum of precipitation (mm) 30 days prior to incubation. Data from Alaska Climate Research Center (2016).
[d] *H. splendens*=0, *P. schreberi*=1.
[e] Alaska paper birch=0, black spruce=1.
[f] Average air temperature (°C) at each of my 6 Murphy Dome weather stations, 30 days prior to incubation.
[g] Average photosynthetic active radiation (μmol/m^2) at each of my 6 Murphy Dome weather stations, 30 days prior to incubation.
[h] 90 days N mineralization rates in the fibric layer, as measured by Melvin et al. (2015).